ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 44 • 1959 • NUMBERS 1-13

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

NEW YORK ZOOLOGICAL SOCIETY

GENERAL OFFICE
30 East Fortieth Street, New York 16, N. Y.

PUBLICATION OFFICE
The Zoological Park, New York 60, N. Y.

OFFICERS

PRESIDENT	VICE-PRESIDENT	SECRETARY	TREASURER
Fairfield Osborn	Laurance S. Rockefeller	George Wall Merck	David H. McAlpin

SCIENTIFIC STAFF:

John Tee-Van ..... *General Director*
Christopher W. Coates.. *Director, Aquarium*

ZOOLOGICAL PARK

Joseph A. Davis, Jr... *Assistant Curator, Mammals*
Grace Davall..... *Assistant Curator, Mammals and Birds*
William G. Conway.. *Curator, Birds*
Herndon G. Dowling. *Associate Curator, Reptiles*
Charles P. Gandal .. *Veterinarian*
Lee S. Crandall..... *General Curator Emeritus*
William Beebe..... *Honorary Curator, Birds*

AQUARIUM

James W. Atz..... *Associate Curator*
Carleton Ray..... *Assistant to the Director*
Ross F. Nigrelli..... *Pathologist & Chairman of Department of Marine Biochemistry & Ecology*
Sophie Jakowska..... *Research Associate in Experimental Biology*
C. M. Breder, Jr..... *Research Associate in Ichthyology*
Harry A. Charipper... *Research Associate in Histology*
Homer W. Smith..... *Research Associate in Physiology*

GENERAL

William Bridges .. *Editor & Curator, Publications*
Sam Dunton ..... *Photographer*
Henry M. Lester.. *Photographic Consultant*

DEPARTMENT OF TROPICAL RESEARCH

William Beebe..... *Director Emeritus*
Jocelyn Crane..... *Assistant Director*
David W. Snow ..... *Resident Naturalist*
Henry Fleming ..... *Entomologist*
John Tee-Van ..... *Associate*
William K. Gregory.... *Associate*

AFFILIATES

L. Floyd Clarke ..... *Director, Jackson Hole Biological Research Station*

SCIENTIFIC ADVISORY COUNCIL

A. Raymond Dochez
Alfred E. Emerson
W. A. Hagan
Caryl P. Haskins
John S. Nicholas

EDITORIAL COMMITTEE

Fairfield Osborn, *Chairman*
James W. Atz
William Beebe
William Bridges
Christopher W. Coates
William G. Conway
Lee S. Crandall
John Tee-Van

Contents

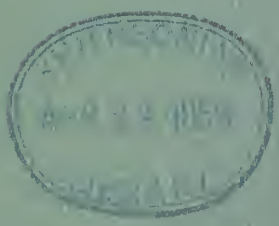
Part 4. December 31, 1959

PAGE

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 44 • PART 1 • MARCH 31, 1959 • NUMBER 1



PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

1

Middle-American Poeciliid Fishes of the Genera *Carlhubbsia* and *Phallichthys*, with Descriptions of Two New Species[1, 2]

DONN ERIC ROSEN

Genetics Laboratory, New York Zoological Society,
and
New York University, New York

&

REEVE M. BAILEY

Museum of Zoology, University of Michigan,
Ann Arbor, Michigan

(Plates I-VI; Text-figures 1-10; Maps 1 & 2)

TABLE OF CONTENTS

INTRODUCTION

THE fishes of the family Poeciliidae (Order Cyprinodontiformes, also known as Cyprinodontida, Cyprinodontes and Microcyprini) are all from the New World and most of the species bear living young. They abound in the fresh and brackish waters of Mexico, the West Indies and northern and eastern South America, but attain their maximum diversity in Central America (Rosen & Gordon, 1953: 1-6). In Middle America three nominal species classified in two genera, *Carlhubbsia* (formerly *Allophallus*) and *Phallichthys*, have heretofore been regarded as closely related. Their many superficial resemblances include the asymmetric twisting or folding, either sinistrally or dextrally, of the external genitalium (gonopodium) of the adult male. This modification is shared by several other genera *(Poeciliopsis, Aulophallus, Phalloptychus* and *Xenophallus)*. On the basis of this one feature, all of them have been grouped as the subfamily Poeciliopsinae (Hubbs, 1926; 1936).

We now find evidence, however, that asymmetry of the gonopodium is not in itself an adequate criterion of the implied phylogenetic relationships of the fishes in the Poeciliopsinae. As in other poeciliids, it is the fine details of the

[1] Much of the material in this paper was included in a thesis presented by Rosen in partial fulfillment of the degree of Master of Science in the Department of Biology, New York University.

[2] This work was supported by a grant from the National Science Foundation to the New York Zoological Society for the project, "A Biological Synthesis of Poeciliid Fishes," Dr. Myron Gordon, New York Zoological Society, Principal Investigator.

gonopodia and characters in the gonopodial suspensoria that provide the most useful criteria in determining relationship. On the basis of new data, *Carlhubbsia* shows affinities to *Quintana* and *Girardinus* (including *Toxus, Glaridichthys, Allodontium* and *Dactylophallus);* these are endemic Cuban fishes with symmetrical gonopodia. *Phallichthys* is closely allied to *Poeciliopsis* (including *Poecilistes*) and *Aulophallus,* but the remaining genera previously associated with them in the Poeciliopsinae are not intimately related. For these reasons the dissolution of the Poeciliopsinae is now recommended.

Materials and Methods

Material.—Most of the specimens used in this study are preserved in the Museum of Zoology of the University of Michigan (UMMZ). Additional specimens are from the collections of the Chicago Natural History Museum (CNHM), Genetics Laboratory of the New York Zoological Society (NYZS-GAF) and the United States National Museum (USNM). In addition to most of the material of *Carlhubbsia* and *Phallichthys* previously recorded, we have had access to numerous and extensive series of the four known species belonging to these genera from Guatemala and Honduras. These were collected largely by the Rev. Gerald Fairweather, Drs. Myron Gordon, Carl L. Hubbs, Laurence C. Stuart and their field associates.

Counts and Measurements.—The methods of counting and measuring are those described by Miller (1948: 8-14) for certain cyprinodontid fishes. *Fin ray counts* include small anterior rays, but in dorsal and anal fins the last ray as counted consists of two elements that are separate to their bases. In the genera studied either one or two anterior dorsal rays are simple, the rest are branched distally. Careful examination under transmitted light is necessary to establish the number of simple rays, and since branching may be delayed, the definitive presence of a single simple ray can be reliably determined only in adults. Caudal ray counts include branched rays plus two. *Scales in lateral series* are counted from the upper angle of the gill aperture to the caudal base at midside. *Body-circumference scales* are counted from about two scale rows before the dorsal origin to an equal distance in front of the pelvic fins. The *vertebral count* includes the urostylar vertebra. *Head angle* is measured with an arm protractor: one arm is placed parallel to the predorsal contour (if flat) or tangential to it (if gently curved); the other is adjusted to coincide with the straight line along the lower surface of the head and the anterior part of the breast (the oblique upward slant of the lower jaw is disregarded). *Standard length* is measured from snout tip to caudal base. *Head length* is taken to the opercular margin. *Diameter of orbit* is established by slipping caliper points within the orbital rim and spreading them gently. All measurements were recorded to tenths of millimeters.

Skeletal Material.—For clearing and staining, alcoholic (ethyl alcohol) specimens were washed briefly in tap water and transferred to 3% solution of KOH in tap water. Ten fish averaging 1½ inches in standard length were placed in 300 cc. of this macerating solution. When the fish became translucent in alkali (4 to 6 weeks at room temperature) enough Alizarin Red S was added to color the solution deep wine red. Within a week the bone and scales became intensely stained, and then all scales were removed. The fish were then placed directly into 50 cc. of glycerine where they cleared in about three days.

In disarticulating a skeleton for study a section of the cleared and stained specimen was first placed in warm 50% KOH solution. The rapid progress of the maceration was watched with a dissecting microscope; when minute bubbles formed, muscle and connective tissues were freed readily from the bone with a dissecting needle. As the bones separated they were transferred to cool tap water to which a few drops of acetic acid had been added to check further disarticulation.

Skeletons or parts thereof were drawn with the aid of a camera lucida. The material (in water or glycerine) was placed in a petri dish and intense reflected light was directed on it from illuminators on each side of the microscope stage. A manipulator fitted with solid glass needles was used to orient specimens. The camera lucida was adjusted to give a distortion-free image on the recording surface by sighting down a hollow tube placed at the geometrical center of the proposed drawing. The specimen was brought into line with the image of the hollow tube that was resting on the recording surface. Relative positions and dimensions of different structures were determined by utilizing the coordinate system of the graph paper employed as a drawing surface.

Preliminary drawings were refined and details added in free-hand from direct observations. The few cleared and stained skeletons were checked for accuracy by comparison with X-rays of series of specimens which were prepared as outlined by Miller (1957).

The distal part of the gonopodium is equipped with a variety of spine-like processes and other

specializations. The terminology here adopted for these structures is that proposed and described by Rosen & Gordon (1953: 18-23). For example, *spines* are specialized bony structures that arise on the ventral (anterior) surface of the distal third of ray 3; *serrae,* if present, are located on the posterior edges of rays 4 or 5, those found proximally on ray 4p function as an anchor for the collagenous tissue sheets that envelop the rays, those found near the distal end of the rays serve as holdfast structures during copulation; the *hook* is a terminal bony segment on ray 3 or ray 4a.

Systematic Account

Genus *Carlhubbsia* Whitley

Allophallus.—Hubbs, 1936: 232 (original description; type species *Allophallus kidderi* Hubbs).

Carlhubbsia.—Whitley, 1951: 67 (replacement synonym of *Allophallus* Hubbs, which name is preoccupied by *Allophallus* Dziedzicki, 1923, in Diptera).

Description.—Body moderately deep and compressed, with distinctly or moderately angulated dorsal and ventral margins, covered with large cycloid scales. *Dorsal fin* typically falcate, often pointed, with 8 to 10, usually 9, rays; the first two rays simple (rarely only the first), other rays bifurcate one or more times (in adult). *Pelvic fin* without fleshy appendages, constantly with 6 rays, the second and third barely prolonged in adult males. *Anal* rays 9 to 11, usually 10. *Gonopodium* permanently folded to form a broad groove on the right side; single series of flat, irregular unpaired serrae on right half of ray 5p extending 8 to 20 segments beyond tip of ray 6; single series of unpaired serrae distally on the lateral margin of left half of ray 5a; single series of unpaired serrae originating distally on left half of ray 4p, tightly grouped into a cluster; distal and subdistal elements of ray 4p that lack serrae extremely slender, reduced or obsolescent; terminal segments of ray 4a widened transversely; ray 3 terminated by minute bony or membranous hook, without consolidated terminal or subterminal segments, right half of ray with row of unpaired broad and flat spines forming ventral wall of groove, left half with minute denticles on subdistal elements; segments of distal half of ray 6 swollen, transversely thickened, those of basal half asymmetrical, the paired elements not side by side; rays 7 and 8 simple, distinctly separated, not converging or in contact along middle of their lengths. *Gonopodial suspensorium* with three gonapophyses; uncini on first two gonapophyses emerging near base of spine, not curved, moderately slender; uncini on third gonapophysis, if present, emerging midway along spine, usually closer to vertebral axis than tip of spine, not curved downward, moderately thickened, rarely slender; uncini of all gonapophyses overlappping one another, forming uncinar plane extending downward and backward at angle of approximately 30 to 45 degrees with horizontal. Dorsal half of primary gonactinostal complex greatly dilated antero-posteriorly, upper edge of complex slightly notched or uniform and unbroken. *Vertebrae* 28 or 29, rarely 30. *Pectoral girdle* somewhat triangular in outline, its longest dimension vertical; four discrete actinosts recessed within posterior margins of scapula and coracoid, not approximating lower margin of coracoid; upper part of cleithrum produced backward above scapula as large spatulate process; posterior edge of coracoid below actinosts produced backward as flat process similar in outline to cleithral process but smaller and variously developed. *Skull* deep and wedge-shaped, with well-developed supraoccipital processes and variously developed epiotic processes; jaws weak, consisting of slender elements with delicate articulations; preorbital (lacrymal) heavily sculptured, with a well-developed process extending backward toward lateral ethmoid; premaxillae and dentaries flattened in front, the paired elements not joined at midline and separated by a distinct tissue space, each with an outer series of movable, compressed or narrow incisor-like teeth in a single, largely transverse row that is weakly indented near midline, and an inner series of minute slender and pointed teeth in a narrow band from 1 to 3 teeth wide. *Intestine* long, coiled, lying largely on right side of coelom. *Peritoneum* dark. *Cephalic canals* rather well developed; supraorbital canal typically with a developed tube connecting pores 2, 3, and 4a, and a postorbital section connecting pores 6 and 7 (Gosline, 1949), sometimes with a third section connecting pores 4b and 5; preopercular canal typically with 7 pores; mandibular canal undeveloped; preorbital canal with 3 or 4 pores in adult. (See Table 1.)

Status.—Carlhubbsia consists of two distinct species, *C. kidderi* (Hubbs) and *C. stuarti,* n. sp. (pages 5-16, and Tables 1-5, 13-19), and probably a third, as yet undescribed because of inadequate material. The genus is confined to the Atlantic drainage of Middle America from the Isthmus of Tehuantepec to southern Guatemala.

Allophallus [=*Carlhubbsia*] *kidderi* was grouped by Hubbs (1936) with *Phallichthys amates* and *P. pittieri* in a subfamily Poeciliopsinae that Hubbs (1924) erected to include all

TABLE 1. COMPARISON OF TWO SPECIES OF *Carlhubbsia*
Measurements are based on adults and are expressed as percent. of standard length

Character	*C. stuarti*	*C. kidderi*
Pectoral rays	13, rarely 14	9 or 10, rarely 11
Preorbital pores (adults)	Usually 4; if 3 with an open groove at ventral end	Usually 3; without groove
Vertebrae	Usually 28	Usually 29
Gonapophyses	With uncini on I and II only	With uncini on I, II and III
Gonopodium:		
Left half of ray 5a	With 15 to 20 terminal serrae	With about 6 terminal serrae
Left half of ray 5p	Continuous with terminal serrae on ray 5a	Obsolescent distally, not continuous with terminal serrae on ray 5a
Tip of ray 4p	With cluster of about 8 serrae	With cluster of 5 serrae
Body circumference scales	23 to 26; infrequently 23	22 to 24; infrequently 24
Dorsal fin	With dusky marginal band	With jet black spot near posterior margin
Vertical bars on body	Well defined	Faint
Dark scale borders	Not well marked	Well defined
Greatest body depth		
Males	39 to 42	27 to 30
Females	37 to 42	29 to 33
Least depth		
Males	22 to 25	14 to 18
Females	21 to 24	16 to 18
Predorsal length		
Males	54 to 58	50 to 54
Females	56 to 59	50 to 53
Preanal length		
Males	54 to 58	48 to 52
Females	64 to 67	57 to 62
Dorsal origin to caudal base		
Males	49 to 54	51 to 55
Females	48 to 51	50 to 54
Anal origin to caudal base		
Males	49 to 55	52 to 54
Females	40 to 42	42 to 45
Head length	29 to 34	25 to 30
Head width	18 to 20	14 to 18
Snout length	8 to 10	5 to 9
Postorbital length of head	11 to 13	9 to 12
Interorbital, bony width		
Males	13 to 15	9 to 10
Females	13 to 14	11 to 13
Dorsal fin, depressed length		
Males	31 to 34	27 to 32
Females	27 to 31	31 to 34
Anal fin, depressed length		
Males	36 to 41	49 to 54
Females	22 to 26	23 to 27
Size (standard length)		
Males	Usually 30-40 mm., largest 45 mm.	Usually 16-21 mm., largest 23 mm.
Females	Usually 35-50 mm., largest 55 mm.	Usually 25-40 mm., largest 51 mm.
Head angle	47° to 51°	37° to 42°

poeciliid genera in which the gonopodium of the adult male is asymmetrical. In his key to this group Hubbs (1936) utilized only the most general features of body form and tooth structure to associate *Carlhubbsia* and *Phallichthys*. He regarded the differences in their gonopodia as being sufficient for generic distinction.

Gonopodial Characters in Carlhubbsia.—The dextral folding of gonopodial rays 3, 4 and 5 in *Carlhubbsia* is accompanied by asymmetric modifications of many individual elements. Each type of segment ornamentation, e.g., spines, hooks and serrae (Rosen & Gordon, 1953), reflects the over-all asymmetry either by serial or unilateral reduction or by fusion and consolidation with adjacent or underlying structures. Thus, the basic architecture of the gonopodium in *Carlhubbsia*, as in other poeciliids with asymmetric gonopodia, is often masked by the superimposed concomitants of folding. In view of the proved value of specialized terminal features of the gonopodium to poeciliid taxonomy, it is of phylogenetic importance to distinguish between the basic pattern of terminal modifications and the asymmetric distortions to which the structure has been subjected.

Ray 3, for example, is bilaterally asymmetrical; the segments of the left half of this ray are simple oblong elements with minute denticles on the subdistal elements, whereas those of the right half are transversely widened with long, flat processes arising from their outer, ventral margins. The latter segments form a continuous ridge, that is, the ventral wall of the gonopodial groove. If this ridge is mechanically flattened, or viewed from above, the proximal section resembles the eccentric groove and the distal section the series of gonopodial spines that commonly occur in bilaterally symmetrical gonopodia in numerous poeciliid genera. The unilateral reduction of an entire series of segments and the distortion of the remaining series on ray 3 tends to obscure the character of the individual segment types. Nevertheless, these structures appear to have arisen in a manner similar to, if not identical with, the eccentric grooves and spines in forms with symmetrical gonopodia. For this reason they are so treated in our taxonomic reassessment.

Consequent to their reduction and consolidation in the evolution of asymmetrical gonopodia, some segments have become isolated from their parent rays and/or subsequently fused to others. The isolated cluster of serrae near the tip of the gonopodium in *Carlhubbsia* is known to arise from ray 4p because early developmental stages show these serrae to be continuous with the proximal elements of this ray. Subsequently the proximal elements are partly obliterated by fusion with the underlying segments of 4a.

The serrae on ray 5 in *Carlhubbsia kidderi* are isolated from the elements of 5p and are consolidated with the underlying segments of 5a; thus, in this species, they might appear as a *de novo* feature of ray 5a. In *C. stuarti*, however, these same serrae, though equally well consolidated with the segments of 5a, are continuous with the elements of 5p, which in this species are persistent. It seems certain, therefore, that these serrae in *Carlhubbsia* originated from a developmental field associated with ray 5p.

Carlhubbsia stuarti, n. sp.

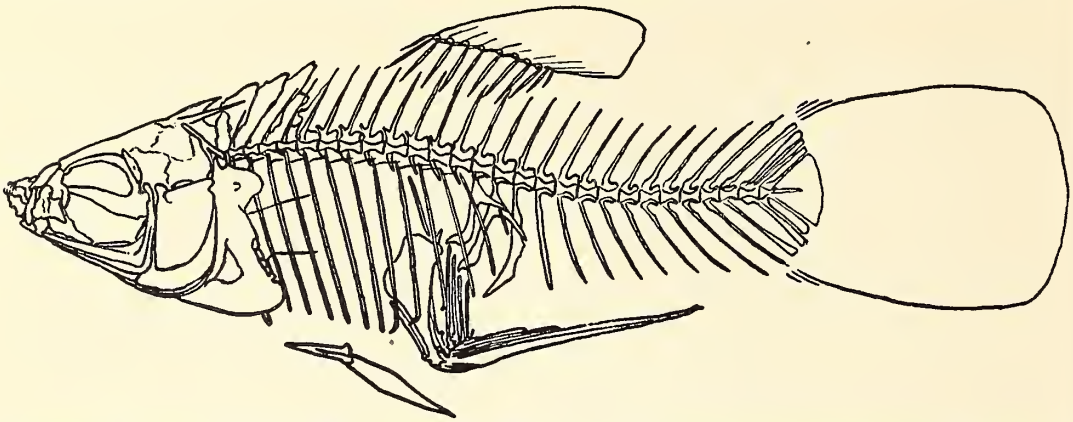
Pl. I; Text-figs. 1, 3, 5

Material.—The holotype (UMMZ 146084) is an adult male, 38.3 mm. in standard length, seined in the Río Polochic at the "playa," about 0.5 km. east of Panzós, Alta Vera Paz, Guatemala, on March 6, 1940, by Laurence C. Stuart. The allotype (UMMZ 172455), an adult female 50.5 mm. long, and 290 other specimens (UMMZ 146078) including half-grown and adult males and females from 29 to 55 mm. long, were taken with the holotype. UMMZ 146093, 5 subadult to adult specimens from 29 to 39.5 mm. long, seined in "El Canal," a diversion of Río Polochic, about 10 km. by river below Panzós, Alta Vera Paz, Guatemala, on March 8, 1940, by Stuart.

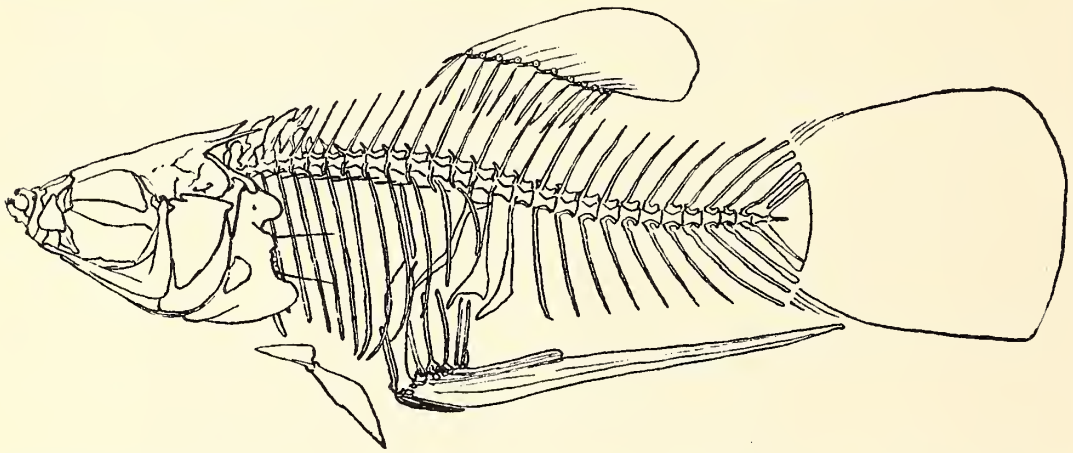
Diagnosis.—A large, deep-bodied, large-headed species of *Carlhubbsia* with 8 to 13 well to moderately developed narrow dusky bars on the side. Dorsal fin rather bluntly rounded anteriorly, projecting beyond succeeding rays to give the fin a falcate outline, with dusky distal band. Gonopodium with series of 15 to 20 terminal serrae on lateral margin of left half of ray 5a, the proximal members usually bidentate, the last member continuous with segments of ray 5p; tip of ray 4p with a cluster of approximately 8 serrae, the subdistal elements of this ray extremely weak but not obsolescent. Gonopodial suspensorium with uncini developed on gonapophyses I and II. Vertebrae 28. Preorbital canal with 4 pores in adult. Scales in lateral series 25 or 26. Body-circumference scales 23 to 26. Pectoral fin rays 13, rarely 14. Head angle 47° to 51°.

For the distinctive features of body and fin form, pigmentation and skeletal morphology, see also Tables 1-3, 13-18.

General Description.—A deep-bodied, robust species with moderately high and angular contours. In adult males the predorsal profile is



TEXT-FIG. 1. Skeleton of adult male *Carlhubbsia stuarti,* n. sp. Compare the following positional and structural features with these items in Text-fig. 2: (a) development of neural plates on anterior trunk vertebrae, (b) position of pelvic girdle, (c) curvature of distal tips of posterior pleural ribs, (d) form of first three sexually modified, attached hemal spines (gonapophyses), (e) relative positions of dorsal and anal fin origins, and (f) length of sexually modified anal fin (gonopodium). Tracing from an X-ray.



TEXT-FIG. 2. Skeleton of adult male *Phallichthys fairweatheri,* n. sp. See caption of Text-fig. 1. Tracing from an X-ray.

flat or slightly arched from nape backward and rises sharply to the dorsal origin; the dorsal and ventral margins of the caudal peduncle also are quite straight but taper rather gradually to the caudal base. In adult females the dorsal profile usually is slightly arched, and rises less abruptly to the dorsal origin; the anterior part of the caudal peduncle is relatively heavier in adult females and its slightly concave dorsal and ventral margins taper more abruptly to the caudal base. In both sexes the head angle measures 47° to 51°. In both sexes the snout is distinctly blunt and the lower jaw rises sharply and obliquely upward instead of outward from its articulation. The male is relatively deeper bodied than the female, and has more angular contours.

The dorsal fin is distinctly falcate in both sexes. The anal fin is broad and fan-shaped. In adult females the dorsal originates closer to caudal base than to snout, and the anal originates slightly behind the vertical from the dorsal origin. In adult males the two fins originate in the same plane as a result of the forward migration of the anal fin during sexual differentiation. The tip of the gonopodium extends posteriorly approximately two-thirds the distance from anal origin to caudal base. In both sexes the caudal fin is broad, subtruncate and only slightly rounded at its dorsal and ventral trailing edges, and is yellow toward the base. There are from 17 to 19 principal caudal rays, most often 17. The pelvic fins are well developed in both sexes

and are bright yellow. In adult females the pelvics are almost as long as the anal, originating on the belly one-half the distance from the anal origin to the edge of the opercle. When folded back the tip of the longest pelvic ray extends to the anal opening or barely overlaps the anal fin origin. In adult males the pelvics originate just anterior to the base of the gonopodium and the longest ray overlaps and extends beyond the bases of modified anal rays 1 and 2. In both sexes the pectoral fins are broadly spatulate and originate well below the midlateral line just behind the opercular margin; they extend obliquely upward and backward to a vertical from the dorsal origin.

The gillrakers on the outer face of the first arch, though well developed, are short and slender; they number 18 to 21.

Two principal pigment patterns on the trunk and caudal peduncle consist of a cross-hatched reticulum, most evident on the dorsum but clearly defined on the sides and venter as well, and a series of as many as 13 to 15 vertical bars superimposed on the reticular network. These bars are most pronounced in adult males. The bars are best developed on the caudal peduncle but extend anteriorly almost to the pectoral base. Each bar is long and slender, extending usually to within one scale row from the middorsal and midventral lines. There is no dark subocular bar or tear drop. In adults of both sexes the distal third of the dorsal fin is dusky. There is a basal band consisting of darkened interradial membranes in the proximal third of the dorsal fin. Between the darkened bands, the middle third of the fin is lighter; anteriorly there is a light sprinkling of melanophores but posteriorly the membranes are clear. Probably this area was colored in life. Other fins are clear, with only a scattering of fine melanophores at their bases.

Skeletal Morphology.—The vertebral axis consists of 28 elements in each of 23 specimens X-rayed, including the holotype. The column is divided approximately in half into a precaudal or trunk series and a caudal series, the division being determined by the first hemal spine. In adult females, the first hemal spine usually emerges from the 15th vertebra. In adult males, the first hemal spine or gonapophysis is on the 14th vertebra. The vertebral axis takes the form of a gentle sigmoid curve, the precaudal section arching upward, especially in large adults. The first cervical vertebra bears no rib, but a pleural rib is present on most of the remaining precaudal vertebrae. The first rib is long and slender and is loosely articulated with the posterodistal margin of the transverse process of the second vertebra; the rib lies against the medial surface of the pectoral girdle. Near the distal end of the rib there lies a free stylet-shaped bone which is expanded proximally and slender distally. This was termed the postcleithrum by Woods & Inger (1957: 247). This bone is well separated from the cleithrum and there is no other bone in series with it. The first 9 or 10 pairs of ribs are long, after which they diminish in size. The last definitive pleural rib usually occurs on the 13th vertebra but a delicate rudimentary rib may occur on the 14th. Minute epipleurals are present on all but the last two or three pleural ribs. In adult males the last five or six ribs are sexually modified. The last three or four are relatively small, extremely slender, and are widely separated at their tips; the tips of the preceding two or three ribs are bent sharply forward toward the pelvic girdle but do not converge. In adult males parapophyses occur on the first and second caudal vertebrae though they are usually quite small and closely applied to the base of the modified hemal spines or gonapophyses. In adult females parapophyses occur on the first two or three caudals. The so-called parapophyses of the more posterior trunk vertebrae, and of the anterior caudals in other species, actually are serially homologous with the transverse processes of the cervical and postcervical elements.

The gonopodial suspensorium of adult males receives a contribution from the vertebral axis of four specialized hemal spines. The first, or ligastyle, is a long, slender rod that originates on the 13th vertebra and migrates forward so that its dorsal margin comes to lie directly beneath thc centrum of the 10th vertebra. The remaining three hemal spines become specialized chiefly by the addition of bony substance to their distal and posterior surfaces and are referred to as gonapophyses. The first two incline forward. Gonapophysis III is either slightly arched forward or essentially vertical. Only gonapophyses I and II bear uncini, which emerge proximally on the spines close to the vertebral centrum; the uncini of the second spine, however, are slightly farther down the shaft and more robust.

The actinosts of the anal fin in the adult male also are specialized and are incorporated into the suspensorial system as the gonactinosts (for general orientation see Rosen & Gordon, 1953: 11-13, Text-figs. 14-16). The first is a short, heavy, blunt rod that inclines sharply forward. It articulates with fin rays 1 and 2. Gonactinosts 2, 3 and 4 are fused to form a single highly complex plate of bone, the primary gonactinostal complex, which supports fin rays 3, 4 and 5. The complex is greatly dilated antero-posteriorly, having a distinct rostral bulge just above the tip

of the first gonactinost. The posterior lateral wings that are produced symmetrically from eccentric positions along either side of the core of gonactinost 4 are rather well developed, particularly at the dorsal third of the complex where they flare broadly. Gonactinost 5 lies embedded in the trough formed by the lateral wings of the primary complex. Gonactinosts 6, 7, 8 and 9 are slender and rod-like; they are closely joined and their tips converge slightly. Actinost 10 of the anal fin becomes much reduced or obsolescent and is incorporated into the base of gonactinost 9 as a tiny sliver of bone. The gonactinosts are firmly anchored to the vertebral axis by means of the ligamentous tissues associated with the ligastyle and the gonapophyses. The primary gonactinostal complex is associated with the ventral margins of the ligastyle and gonapophysis I. The tip of gonapophysis II interdigitates with gonactinosts 7 and 8. The tip of gonapophysis III curves forward toward the base of the final gonactinost.

The gonopodium in the adult male consists of the produced and modified rays 3, 4 and 5 of the anal fin. Together these three rays are folded to form a broad groove along the right side of the gonopodium. When held at rest, i.e., pointed caudally, ray 3 forms the ventral border, ray 5 the dorsal border and ray 4 the lateral wall of the dextral groove. The paired halves of ray 3 are segmented to the tip of the fin and are never consolidated. On ray 3 there is a series of from 15 to 20 long spinous processes which are followed distally by 5 to 8 simple terminal segments. The spines are present on the right half of the ray only; they form the ventral margin of the dextral groove. The terminal elements of ray 4a are expanded dorso-ventrally, and their segmentation axes are at right angles to the long axis of the ray. The elements of ray 4p are extremely delicate terminally but are never obsolescent. Its sinistral branch terminates in a cluster of 7 or 8 subtriangular serrae which face outward, away from the gonopodial groove on the convex surface of the permanently folded fin. On ray 5p there is a single series of unpaired retrorse distal serrae; they originate on the sinistral portion of this ray and are fused with the underlying segments of 5a. The unpaired serrae are subtriangular, approximately 15 in number. Several of the proximal members of this series are bidentate. Proximally on 5p, just below the tip of ray 6, there is a series of 5 to 10 minute retrorse serrae. The elements of ray 5a are flattened dorso-ventrally but are otherwise simple; they extend to the tip of the fin. The tips of all the rays became slender distally but show no significant displacement from their original axes; they are grouped rather tightly together and are never separated by distinct tissue spaces. The gonopodium as a whole has a distinctly acuminate profile and is terminated by a rigid, hook-like cap of tough membranous tissue.

Relationship.—This species is clearly referable to the genus *Carlhubbsia* on the basis of details in the gonopodium and gonopodial suspensorium (see Text-figs. 3 and 5 and discussion on pp. 29-32). It may be separated readily from the only other known species, *C. kidderi,* by the characters listed in the diagnoses and in Table 1. The two species are allopatric, being separated geographically by a linear distance of approximately 45 miles in drainages which empty on opposite sides of the Yucatan Peninsula (Map 1). The origin and relationship of the members of the genus *Carlhubbsia* are discussed below (pp. 35-39).

Habitat.—At the two known localities Dr. Stuart recorded the water as muddy, but not excessively so, the bottom as sandy mud, the current as moderate and as slow, the temperature as warm, and the depth of capture as up to 5 feet.

Range.—Carlhubbsia stuarti is recorded only from the drainage of Río Polochic, Guatemala (Map 1). Robert R. Miller, in 1946, found this species to be common also in Lake Izabal, the terminus of Río Polochic.

Etymology.—This species is named in honor of Dr. Laurence C. Stuart in recognition of his efforts in collecting this and many other species of freshwater fishes in Guatemala during the past quarter-century.

Carlhubbsia kidderi (Hubbs)

Pl. II; Text-figs. 3, 5

Allophallus kidderi. — Hubbs, 1936: 232-238 (original description; Río Champotón, Campeche, Mexico). Scrimshaw, 1944: 182 (superfetation). Scrimshaw, 1945: 234-237 (viviparity). Scrimshaw, 1946: 21-22 (egg size).

Aulophallus kidderi.—Scrimshaw, 1945: 239-241 (lapsus for *Allophallus*).

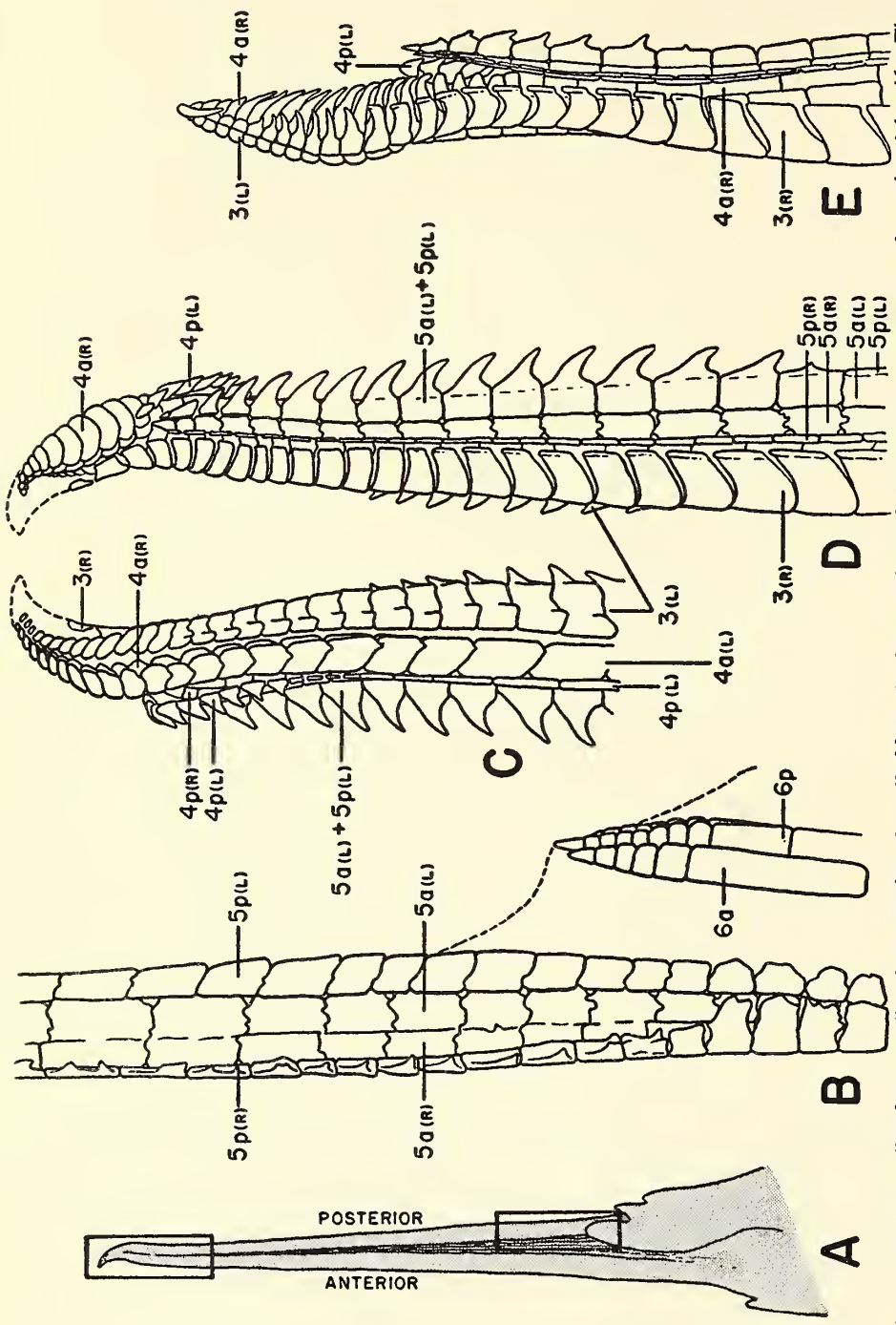
Carlhubbsia kidderi.—Whitley, 1951: 67 (replacement synonym for *Allophallus*). Rosen & Gordon, 1953: 2, 28 (mechanics of gonopodium; reference by generic name only).

Material.—Río Champotón drainage (Campeche, Mexico): UMMZ 102206 (71 hf.-gr., ad. males and females, 16 to 40 mm. standard length), Río Champotón, at Janateya, 7 leagues east of Champotón, July 9, 1932, E. P. Creaser and A. S. Pearse; UMMZ 102199 (holotype of

Map 1. Lower Middle America giving distribution by record stations for the forms of *Carlhubbsia* and *Phallichthys*.

Allophallus kidderi Hubbs, 16 mm.) and UMMZ 102200 (3 subad., 11 to 16 mm.), Río Champotón, about 11 miles from mouth, July 11, 1932, E. P. Creaser.

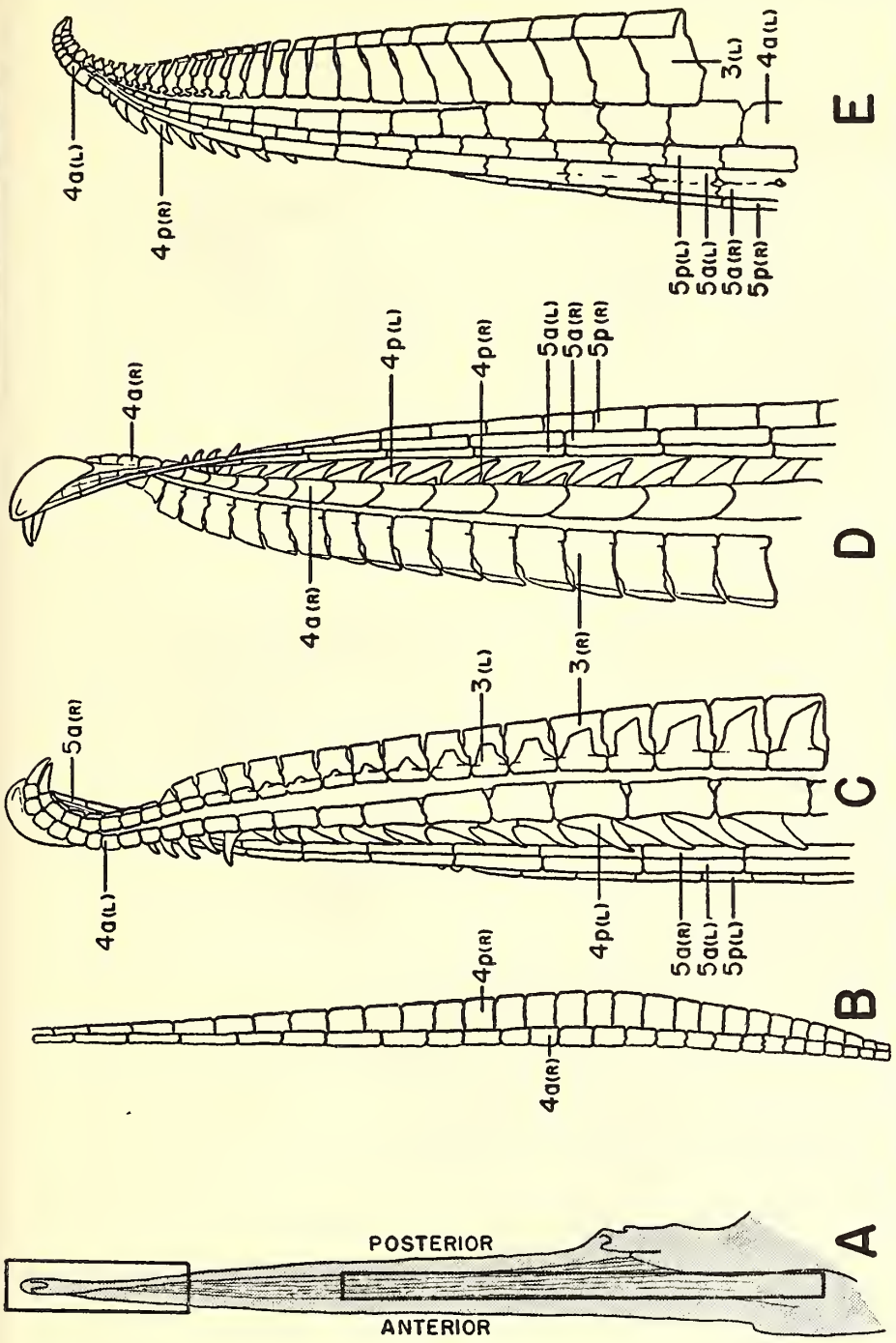
Río San Pedro de Mártir drainage (El Petén, Guatemala): UMMZ 144206 (4 subad., 9 to 32 mm.), Laguna de Yalác, in course of Río San Pedro, about 6 leagues by river (easterly of El Paso de los Caballos), March 17, 1935, Carl L. Hubbs and Henry van der Schalie; UMMZ 144204 (185 hf.-gr., ad. males and females, 16 to 51 mm.), Laguna de Yalác, in course of Río San Pedro, about 6 leagues by river above El Paso de los Caballos, March 16, 17, 1935, Hubbs and van der Schalie; UMMZ 144205 (one ad. female, 39 mm.), Río San Pedro, about ¼ mile below Laguna de Yalác, about 6 leagues by river east of El Paso de los Caballos, March 18, 1935, Hubbs and van der Schalie; UMMZ 144201 (28 hf.-gr. to ad., 17 to 42 mm.), north end of Laguna Perdida, 6 leagues south of El Paso de los Caballos, March 6, 7, 1935, Hubbs and van der Schalie; UMMZ 144202 (138 yg. to ad., 13 to 34 mm.), lagoon-like arm of Río San Pedro at El Paso de los Caballos, March 10-14, 1935, Hubbs and van der Schalie; UMMZ 144203 (94 hf.-gr. to ad. males and females, 16 to 35 mm.), lateral waters of Río San Pedro at Desempeño, just below El Paso de los Caballos, March 12, 1935, Hubbs; UMMZ 144207 (two ad. females 26 and 32 mm.), Río San Pedro, at Mactún Rapids, about



TEXT-FIG. 3. Details of gonopodia of the species of *Carlhubbsia*. A. Gonopodium of *C. stuarti*, n. sp., as seen from the right side. The terms *posterior* and *anterior* refer to orientation of the unmodified rays of the anal fin of juveniles and females as they extend downward; with specialization of the anal fin as a gonopodium, the rays now extend backward directly underneath the male's body. Thus oriented, the originally posterior margin of each ray is dorsal and the anterior one ventral. (See also Text-fig. 1.) B. Proximal portions of rays 5 and 6 of the gonopodium of *C. stuarti*, n. sp., as seen from the right side. 5a (R) = right half of anterior branch of ray 5; 5p (L) = left half of posterior branch of ray 5; etc. This portion of the gonopodium is enclosed by the rectangle at the base of the fin in A. C. Distal tip of the gonopodium of *C. stuarti*, n. sp., as seen from a left three-quarter view to reveal marginal structures. D. Distal tip of the gonopodium of *C. stuarti*, n. sp., as seen from a right three-quarter view. This portion of the gonopodium is enclosed by the rectangle at the tip of the fin in A. E. Distal tip of the gonopodium of *C. kidderi* (Hubbs), as seen from a right three-quarter view.

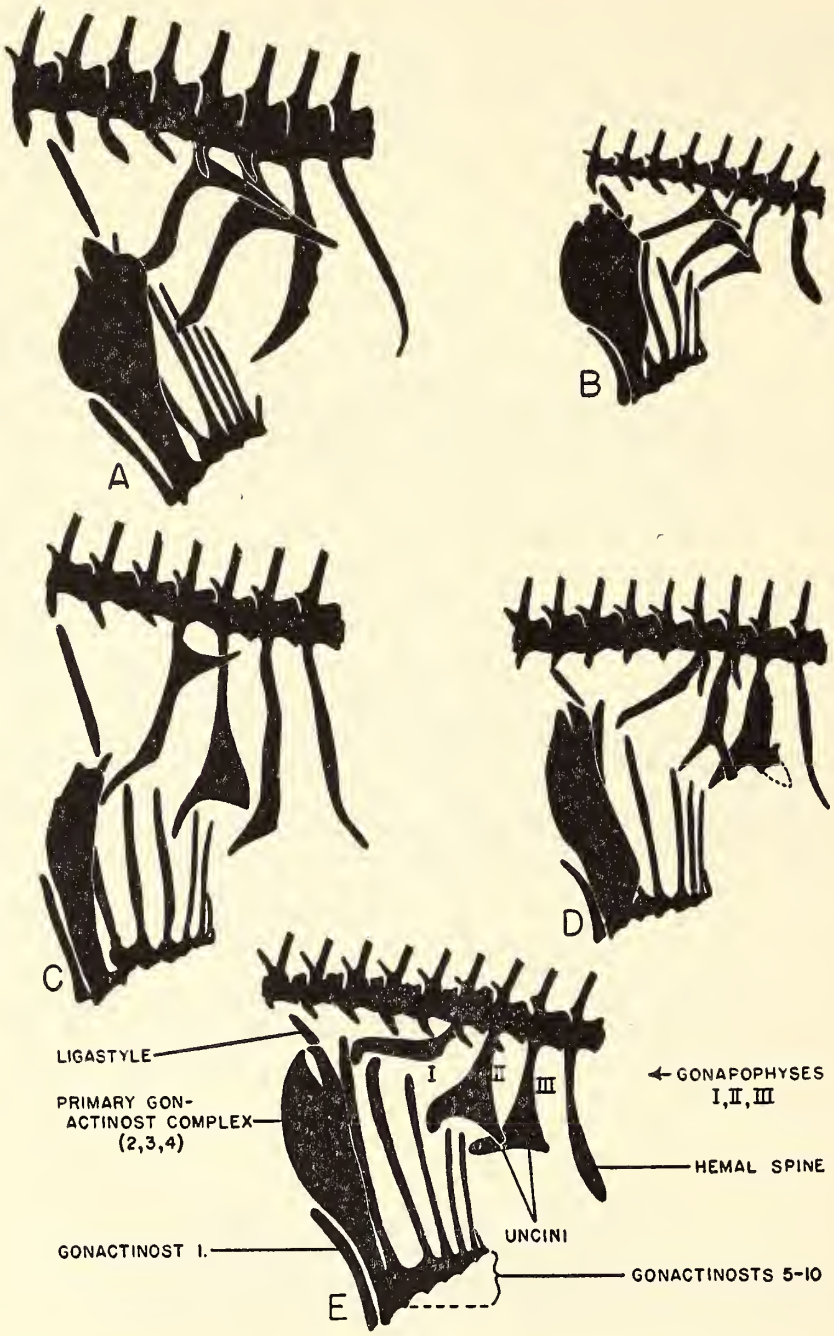
60 miles by stream below El Paso de los Caballos (about two-thirds distance from Laguna de Yalác to Mexican border), March 20, 1935, van der Schalie.

Río de la Pasión drainage (Guatemala): UMMZ 144211 (5 ad. females, 27 to 31 mm.), upper Río de la Pasión (=Río Chajmayic) at Ceiba (about 2½ miles by river below Seból), Alta Vera Paz, April 13, 1935, Hubbs; UMMZ 144212 (one ad. female, 41 mm.), mouth of first arroyo tributary to upper Río de la Pasión, from east below Arroyo San Simón, Alta Vera



TEXT-FIG. 4. Details of gonopodia of the forms of *Phallichthys*. A. Gonopodium of *P. fairweatheri*, n. sp., as seen from the right side. For terminology and orientation, see caption of Text-fig. 3. B. Proximal portion of ray 4 of the gonopodium of *P. fairweatheri*, n. sp., as seen from the right side, showing ridge-like unit formed from the high and laterally compressed segments of posterior branch. This portion of the gonopodium is enclosed by the rectangle at the base of the fin in A. C. Distal tip of the gonopodium of *P. fairweatheri*, n. sp., as seen from the left side, slightly unfolded longitudinally to reveal structural details. D. Distal tip of the gonopodium of *P. fairweatheri*, n. sp., as seen from the right side, slightly unfolded as in C. This portion of the gonopodium is enclosed by the rectangle at the tip of fin in A. E. Distal tip of the gonopodium of *P. amates amates* (Miller), as seen from the left side.

Paz, April 16, 1935, Hubbs; UMMZ 144213 (157 yg. to ad. males and females, 6 to 40 mm.), flooded mouth of Arroyito Jolomáx, opposite (south) El Cambio, El Petén, April 19, 1935, Hubbs; UMMZ 144214 (one ad. female, 25 mm.), Río de la Pasión, at Tres Islas, near southern border of El Petén, April 20, 1935, Hubbs, van der Schalie and Taintor; UMMZ 144200 (103 hf.-gr. to ad. males and females, 9 to 32 mm.), Laguna de Eckibix (extreme west end), in savanna region southeast of Santa Ana, El Petén, February 26, 1935, Hubbs; UMMZ



TEXT-FIG. 5. Gonopodial suspensoria in the forms of *Carlhubbsia* and *Phallichthys*. For orientation, see Text-figs. 1 and 2. Parapophyses, which are not labelled, are small sinuous processes emerging near the bases of gonapophyses I and II in A, D, and E. A. *Carlhubbsia stuarti*, n. sp. B. *C. kidderi* (Hubbs). C. *Phallichthys fairweatheri*, n. sp. D. *P. amates amates* (Miller). E. *P. amates pittieri* (Meek).

144199 (14 hf.-gr. to ad. males and females, 11 to 27 mm.), Laguna de Eckibix (north shore), southeast of Santa Ana, El Petén, February 26, 1935, Hubbs; UMMZ 144198 (8 yg. to ad. males and females, 15 to 28 mm.), Laguna de Eckibix (south shore), southeast of Santa Ana, El Petén, February 26, 1935, Hubbs and van der Schalie; UMMZ 144215 (265 yg. to ad. males and females, 8 to 41 mm.), Arroyo San Martín, close to mouth into Río de la Pasión, east of Sayaxché, El Petén, April 22, 1935, Hubbs, van der Schalie, and Taintor; UMMZ 144216 (73 ad. females, 21 to 38 mm.), lowest mile of Arroyo de Petexbatúm, at and above Sayaxché, El Petén, April 23, 1935, Hubbs; UMMZ 144209 (26 hf.-gr. to ad. males and females, 14 to 35 mm.), Arroyo Subín, at Trinidad, about two miles east (above) Santa Teresa, south of La Libertád, El Petén, April 2, 1935, Hubbs and van der Schalie; UMMZ 144208, 144219, and 144210 (49 yg. to ad. females, 12 to 39 mm.), Arroyo Subín, Santa Teresa, about 13 miles south of La Libertád, El Petén, April 2-3, 1935, Hubbs, van der Schalie and Taintor; UMMZ 144218 (41 yg. to ad. males and females, 11 to 42 mm.), Arroyo Subín, tributary to Río de la Pasión in small bay connected with stream, beside third rapids (about 2½ miles from mouth), El Petén, April 25, 1935, Hubbs; UMMZ 144217 (127 yg. to ad. males and females, 12 to 39 mm.), Arroyo Subín, at second rapids (about two miles) above mouth into Río de la Pasión, El Petén, April 25, 1935, Hubbs.

Diagnosis.—A small, moderately deep-bodied, small-headed species of *Carlhubbsia* with 4 to 6 variably developed, narrow dusky bars on the side. Dorsal fin pointed, more or less falcate, with a conspicuous black blotch on the distal half of the posterior 3 or 4 rays. Gonopodium with series of approximately 6 terminal serrae on lateral margin of left half of ray 5a, left half of ray 5p becoming obsolescent 4 or 5 segments proximal to serrae; tip of ray 4p with cluster of 5 serrae, the subdistal elements of this ray extremely weak or obsolescent. Gonopodial suspensorium with uncini developed on gonapophyses I, II and III, those on III frequently weakly developed. Vertebrae usually 29, rarely 28 or 30. Preorbital canal with three pores in large specimens, or undeveloped and represented by an open groove in small males. Scales in lateral series 26 or 27. Body-circumference scales 22 to 24. Pectoral fin rays 9 to 11, usually 10. Head angle 37° to 42°.

The distinctive features of body form are presented in Tables 4 and 5, and the two known species of *Carlhubbsia* are contrasted in Table 1.

Range.—*Carlhubbsia kidderi* is known to occur in the Río Champotón, Campeche, Mexico, and in the drainages of Río San Pedro de Mártir, El Petén, Guatemala, and Río de la Pasión, Alta Vera Paz and El Petén, Guatemala (Maps 1 and 2). These latter rivers flow into the Río Usumacinta, and it is predictable that when this system is better explored ichthyologically the known range of *C. kidderi* will be substantially increased. During the Fifth Carnegie Institution-University of Michigan Expedition to El Petén, in 1935, Drs. Carl L. Hubbs and Henry van der Schalie failed to take this species in Laguna de Petén or other adjacent disjunct waters of the area, but did collect it in Laguna Perdida and Laguna de Eckibix (Map 2).

Habitat.—The environment where this species was found in El Petén by Drs. Carl L. Hubbs and Henry van der Schalie in 1935 indicates a broad variation in habitat tolerance. To judge by the conditions prevailing where especially large samples were obtained, however, *Carlhubbsia kidderi* prefers quiet to slow-moving water; a bottom composed of soft, flocculent silt, muck or soft mud, or organic litter, with or without some gravel or rocks; moderate to dense vegetation; and shallow shore areas or protected lagoons. At most stations the water was warm or hot and varied from clear to roily or turbid.

Remarks.—To the description of *Carlhubbsia kidderi* by Hubbs (1936: 234, items *5d* and *6d* of key), we make the following corrections and additions: In *5d,* the first sentence reads ". . . rather compressed but pointed incisors, not forming an even cutting edge . . ." Since the teeth are pointed, not chisel-like, the cutting edge is in a sense irregular. However, the tips of the teeth form a straight rather than an irregular or zigzag cutting edge. Also, the teeth of the outer series in *kidderi* originate on the same dental margin and lie side by side, in strong contrast to the arrangement of this series in such species as *Girardinus metallicus* in which the teeth originate on a broad dental ridge, overlap one another shingle fashion, and form a complex cutting edge. In *6d,* the sixth sentence ends ". . . segments of left side only [ray 5a], modified into flat serrae proximal to and partly opposite the serrae of ray 4." This statement should be omitted and the following incorporated at the beginning of the seventh sentence: "Left half of ray 5p becoming obsolescent and merging with 5a at the level of the 12th to 18th segment (counting apico-basally on 5a) and reappearing on the 8th or 9th element of ray 5a as 5 or 6 flat serrae that are fused to the underlying segments; right half of ray 5p made up of delicate, almost thread-like segments, extending unbroken

TABLE 2. PROPORTIONAL MEASUREMENTS OF TEN ADULT MALES OF *Carlhubbsia stuarti*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	UMMZ 146078						Holotype UMMZ 146084	UMMZ 146078		
Standard length (mm.)	45.3	44.1	41.1	38.7	38.5	38.5	38.3	38.2	37.6	34.0
Body, greatest depth	411	408	401	401	403	400	405	424	404	388
Caudal peduncle, least depth	232	238	231	235	231	231	248	233	226	218
Dorsal origin to snout tip	548	556	557	566	569	571	574	560	564	547
Anal origin to mandibular symphysis	545	553	564	576	558	564	574	571	572	573
Dorsal origin to caudal base	521	528	513	517	499	512	535	518	516	488
Anal origin to caudal base	550	522	511	509	525	512	522	531	513	491
Head length	300	306	292	310	301	299	313	314	317	312
Head width	188	197	187	194	195	187	188	194	200	188
Snout length	88	98	85	90	81	91	91	92	82	88
Orbit length	106	113	109	109	106	109	110	113	120	118
Postorbital length of head	115	120	122	127	119	122	117	118	128	118
Orbit to angle of preopercle	46	57	54	52	42	49	60	56	51	47
Interorbital, bony width	132	136	131	142	143	143	144	136	144	132
Mouth, over-all width	88	95	95	90	91	93	97	97	106	88
Dorsal fin, depressed length	311	318	326	318	317	311	339	340	319	312
Anal fin, depressed length	373	365	380	390	390	390	399	393	396	409
Caudal fin length	364	365	389	377	390	377	397	398	391	406
Pectoral fin length	269	268	292	287	273	278	292	288	290	294
Pelvic fin length	212	200	219	209	221	208	227	223	213	206

TABLE 3. PROPORTIONAL MEASUREMENTS OF TEN FEMALES OF *Carlhubbsia stuarti*
The smallest specimen is a juvenile; the others are adult. Proportions are expressed in thousandths of the standard length. See text for source of specimens.

Measurement	UMMZ 146078			Allotype UMMZ 172455	UMMZ 146078					
Standard length (mm.)	51.8	51.5	50.6	50.5	49.5	45.3	44.3	39.1	38.5	31.4
Body, greatest depth	396	394	377	396	398	382	399	402	416	420
Caudal peduncle, least depth	224	214	217	220	222	221	219	223	223	236
Dorsal origin to snout tip	570	583	583	584	572	559	576	563	571	576
Anal origin to mandibular symphysis	657	656	646	669	642	654	652	665	665	669
Dorsal origin to caudal base	496	491	486	489	495	497	485	499	509	494
Anal origin to caudal base	413	414	421	408	404	408	420	420	410	398
Head length	294	291	292	297	291	298	305	312	312	338
Head width	183	184	192	178	182	185	187	192	200	194
Snout length	87	87	81	91	89	88	90	92	90	96
Orbit length	98	101	97	103	105	97	108	105	112	118
Postorbital length of head	118	115	119	119	113	117	115	128	127	143
Orbit to angle of preopercle	58	52	55	55	57	44	56	54	60	64
Interorbital, bony width	133	132	132	135	133	135	129	141	140	140
Mouth, over-all width	87	87	89	93	89	88	86	95	93	89
Dorsal fin, depressed length	276	276	294	287	283	287	282	299	301	309
Anal fin, depressed length	224	221	229	230	228	232	244	253	244	264
Caudal fin length	338	332	346	348	354	336	363	361	379	389
Pectoral fin length	251	252	257	261	248	252	262	263	262	274
Pelvic fin length	193	194	207	202	198	201	194	205	208	210

TABLE 4. PROPORTIONAL MEASUREMENTS OF TEN ADULT MALES OF *Carlhubbsia kidderi*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

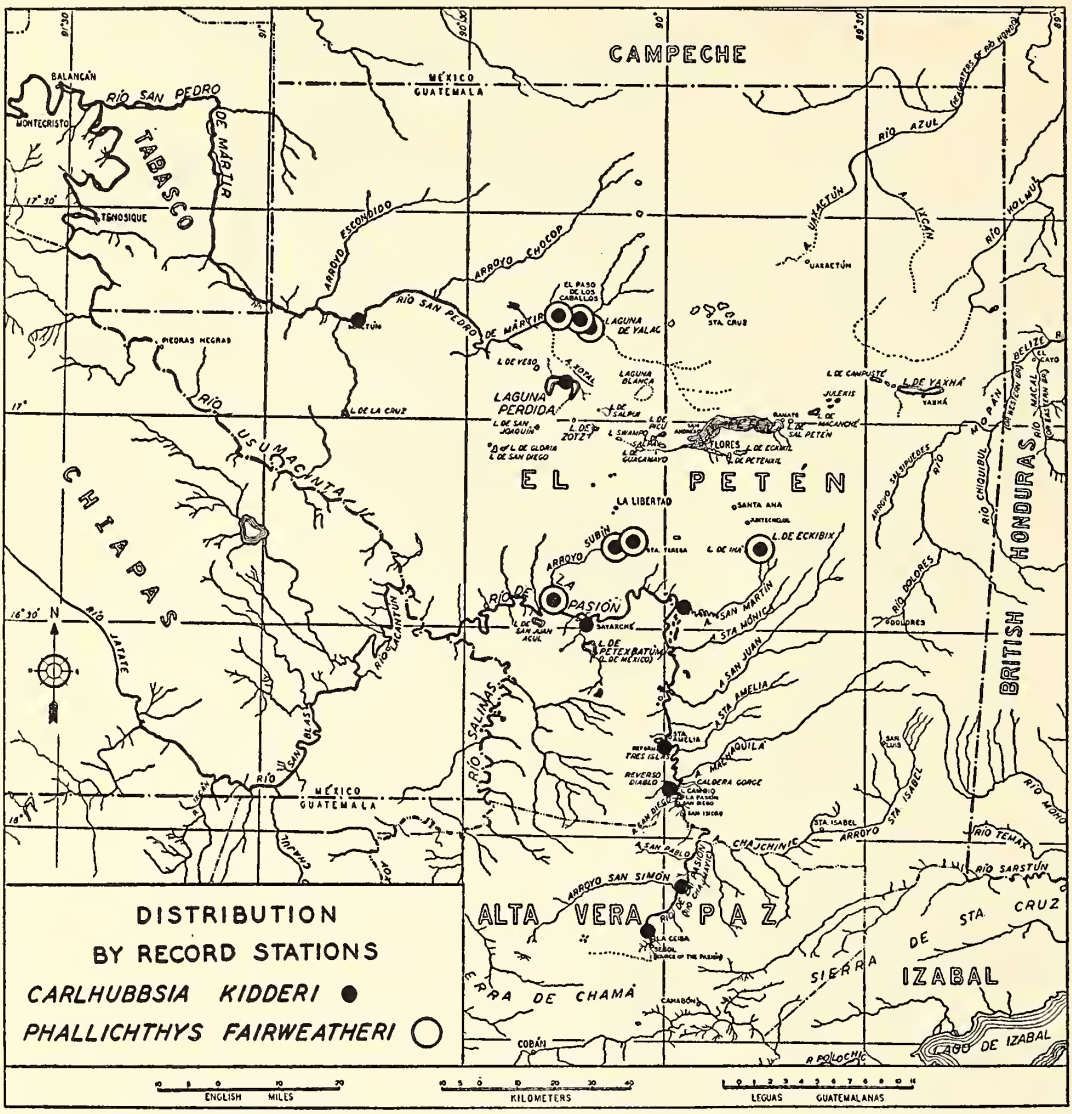
Measurement	UMMZ 144203									
Standard length (mm.)	20.5	20.0	19.7	19.7	19.3	18.0	18.0	16.5	16.5	16.0
Body, greatest depth	288	295	299	299	295	289	289	273	273	275
Caudal peduncle, least depth	171	175	173	173	176	167	167	145	164	156
Dorsal origin to snout tip	512	515	528	503	503	528	506	533	527	525
Anal origin to mandibular symphysis	488	500	508	497	482	500	489	515	485	500
Dorsal origin to caudal base	522	525	523	513	518	528	533	515	515	550
Anal origin to caudal base	522	520	528	533	518	528	533	539	521	538
Head length	278	275	284	274	269	256	261	273	273	288
Head width	141	150	147	147	145	139	139	145	152	138
Snout length	68	75	76	66	62	56	56	61	73	56
Orbit length	107	105	107	102	104	100	106	103	103	100
Postorbital length of head	112	105	107	102	104	100	106	91	97	94
Orbit to angle of preopercle	49	55	66	51	52	56	56	42	42	50
Interorbital, bony width	93	90	91	91	93	94	94	91	91	94
Mouth, over-all width	83	90	86	86	78	83	89	79	85	81
Dorsal fin, depressed length	293	320	...	284	285	306	306	...	291	275
Anal fin, depressed length	488	540	528	533	518	528	528	533	533	525
Caudal fin length	...	...	350	...	337	...	...	...	...	...
Pectoral fin length	...	...	...	...	...	...	...	...	...	...
Pelvic fin length	195	210	203	203	202	194	194	182	200	188

TABLE 5. PROPORTIONAL MEASUREMENTS OF TEN ADULT FEMALES OF *Carlhubbsia kidderi*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	UMMZ 144203									
Standard length (mm.)	34.5	33.0	32.9	32.0	31.7	31.4	30.1	28.9	28.5	27.9
Body, greatest depth	319	321	304	309	322	296	312	308	326	315
Caudal peduncle, least depth	174	179	170	169	170	172	179	166	179	172
Dorsal origin to snout tip	522	512	508	513	514	513	518	519	519	523
Anal origin to mandibular symphysis	609	597	605	609	603	576	608	578	618	595
Dorsal origin to caudal base	516	518	538	516	521	513	508	522	502	527
Anal origin to caudal base	452	439	432	447	426	436	429	443	425	444
Head length	290	288	289	294	293	293	286	291	298	287
Head width	157	164	158	159	155	162	166	173	175	165
Snout length	84	82	85	84	85	86	80	87	88	82
Orbit length	107	112	109	109	110	118	113	111	119	108
Postorbital length of head	110	112	112	113	110	115	110	97	105	100
Orbit to angle of preopercle	52	55	52	56	54	51	56	48	56	50
Interorbital, bony width	113	118	116	119	117	115	116	114	123	111
Mouth, over-all width	90	91	88	81	91	89	86	87	91	86
Dorsal fin, depressed length	...	...	334	...	...	...	309	311	...	...
Anal fin, depressed length	258	...	243	...	...	...	233	270	...	...
Caudal fin length	...	...	256	...	...	...	...	349	...	...
Pectoral fin length	235	...	243	...	...	...	...	221	...	...
Pelvic fin length	194	182	198	191	192	188	196	194	193	190

to within 4 or 5 segments of the tip of ray 5a; . . ." (See discussion on p. 5).

The following account of the pigmentary characters of living *Carlhubbsia kidderi* is taken from original notes made by Carl L. Hubbs in 1935 in El Petén, Guatemala. Coloration of adult male: Dorsal fin [with] bright orange yellow. Caudal peduncle bright yellowish, becoming almost orange above anal fin. Trunk proper without yellow. Anal fin yellow; pelvic orange-yellow. Some males show a reduction in the amount of yellow pigment on body and



MAP 2. El Petén, Guatemala, and adjacent areas showing collection localities in Guatemala for *Carlhubbsia kidderi* and *Phallichthys fairweatheri*. The base map was compiled by Carl L. Hubbs and Henry van der Schalie in 1937.

fins. Coloration of adult female: Olive, with silvery luster on sides of belly; golden amber elsewhere. Dark bars weak, barely evident. Dorsal fin orange-amber, jet black toward posterior outer angle, dusky toward upper margin, bright clear yellow-amber just before black spot. Some orange amber on other fins, especially anal and pelvic.

GENUS *Phallichthys* HUBBS

Phallichthys.—Hubbs, 1924: 10 (type species, by original designation, *Poeciliopsis isthmensis* Regan, a subjective synonym of *Phallichthys amates pittieri* Meek).

Description.—Body deep, moderately compressed, with angulated dorsal and ventral margins, covered with large cycloid scales. *Dorsal fin* rounded, with 8 to 11 rays, the first one or two simple, the other rays bifurcate one or more times (in adult). *Pelvic fin* without fleshy appendages, constantly with 6 rays, the second and third somewhat prolonged in adult males. *Anal rays* 9 or 10. *Gonopodium* permanently folded to the right or left to form a broad groove; ray 5p modified as laterally compressed knife-like ridge forming dorsal wall of groove on left (in sinistral species) or right (in dextral species) half; ray 4p high, laterally compressed and ridge-

like proximally, with row of unpaired distal serrae on right (in sinistral species) or left (in dextral species) half, sometimes with 5 or 6 minute serrae on right half in dextral species; ray 3 with row of unpaired, broad, flat and moderately incurved spines forming ventral wall of groove on left (in sinistral species) or right (in dextral species) half, without consolidated terminal or subterminal segments; segments of distal half of ray 6 swollen, transversely thickened, those of basal half asymmetrical, the paired elements not side by side; rays 7 and 8 distinctly separated, not converging or in contact along middle of their lengths. *Gonopodial suspensorium* with three gonapophyses; uncini on first gonapophysis, if present, emerging near base of spine just below vertebral centrum; uncini of second and third gonapophyses always situated distally on spines to form broad swellings, the tips arching ventrally. Primary gonactinostal complex long, slender, narrow antero-posteriorly. *Vertebrae* usually 28 or 29, rarely 27 or 30. *Pectoral girdle* somewhat triangular in outline, its longest dimension vertical; four discrete actinosts recessed within posterior margins of scapula and coracoid, not approximating lower margin of coracoid; upper part of cleithrum produced backward above scapula as large spatulate process; posterior edge of coracoid below actinosts produced backward as flat process similar in outline to cleithral process but smaller. *Skull* deep and wedge-shaped, with well developed supraoccipital processes and variously developed epiotic processes; jaws weak, consisting of slender elements with delicate articulations; preorbital (lacrymal) little sculptured, subtriangular in outline; premaxillae and dentaries flattened in front, the paired elements not joined at midline and separated by a distinct tissue space, each with an outer series of movable, compressed or narrow incisor-like teeth in a single, largely transverse row that is weakly indented near midline, and an inner series of minute pointed teeth in a band of variable breadth. *Intestine* long, coiled, lying largely on right side of coelom. *Peritoneum* dark. *Cephalic canals* rather poorly developed; supraorbital canal variously incomplete, sometimes reduced to short, postorbital tube connecting pores 6 and 7 (Gosline, 1949), sometimes, especially in large adult females, with short section of tube above eye connecting pore 3 with pores 2 and/or 4a; preopercular canal typically with 7 pores; mandibular canal never developed; preorbital canal with 3 pores in adult, often with two pores and open groove in subadults.

Status.—Phallichthys has previously been associated closely with *Carlhubbsia* [as *Allophallus*] (Hubbs, 1936). Despite strong superficial resemblance between these genera, we find compelling evidence, especially in the comparative morphology of the gonopodium and the gonopodial suspensorium, to regard *Carlhubbsia* as representative of a distinct phyletic line including also the Cuban endemic genera *Girardinus* and *Quintana,* whereas the relatives of *Phallichthys* are to be found in the *Poeciliopsis* complex. The common presence of a folded gonopodium in *Phallichthys* and *Carlhubbsia* is interpreted as the product of parallel evolution. As discussed hereinafter, the nominal subfamily Poeciliopsinae (Hubbs, 1924) based on this supposedly monophyletic feature is in need of re-evaluation.

Phallichthys includes three known allopatric forms which occur on the lowlands of the Atlantic slope of Middle America, ranging from western Panama to northern Guatemala and British Honduras. The distinctive northern species, *P. fairweatheri,* is sympatric with *Carlhubbsia kidderi.* As in the species of *Carlhubbsia* the gonopodial groove in *P. fairweatheri* is dextral. The forms with sinistral gonopodia, *pittieri* from western Panama and Costa Rica and *amates* from northern Honduras and eastern Guatemala, are well separated geographically but are strikingly similar in general appearance and in most diagnostic characters (Henn, 1916; Hubbs, 1924 and 1926). These two are herein treated as subspecies of *P. amates.*

Both *Phallichthys amates* and *P. fairweatheri* have been maintained in the laboratory. As might be anticipated from their body form, they are not agile swimmers, and they progress by means of a series of short, hesitant dashes. In aquaria they are almost exclusively bottom feeders and are rather "timid." An effort was made to hybridize *P. fairweatheri* from British Honduras with a commercial stock of *P. amates.* Six pairings, *amates* ♂ × *fairweatheri* ♀, and four pairings, *fairweatheri* ♂ × *amates* ♀, were uniformly unsuccessful in producing offspring, although homotypic *amates* and *fairweatheri* matings set up at the same time did yield offspring. Each heterotypic pair was isolated in a five-gallon aquarium; the pairings were maintained for eight months (December to July) before being abandoned because of mortalities among the *P. fairweatheri.* Three of the direct and two of the reciprocal matings had been set up in a constant-temperature room with a controlled 12-hour light cycle. Whether or not *fairweatheri* and *amates* are fully isolated reproductively can only be determined by additional experimental matings under a wider variety of aquarium conditions. It is not known whether males of one species actually attempted to copulate with fe-

TABLE 6. COMPARISON OF THE SPECIES OF *Phallichthys*
Measurements are from adults and are expressed as percent of standard length

Character	*P. amates*	*P. fairweatheri*
Scales in lateral series on body	26 or 27	22 to 24
Anal fin rays	Usually 9	Usually 10
Pectoral fin rays	Usually 12 or 13	Usually 11
Vertebrae	Usually 29	Usually 28
Fleshy pad on lower lip	Absent	Developed
Subocular dark bar	Well developed; extends more downward than backward	Poorly defined; extends more backward than downward
Last two anal rays of adult male	Largely black	Tips only black
Margin of anal fin of adult female	Light	Dark
Vertical bands on body of adult female	Weak or absent	Several on urosome
Dark markings on lower side of adult female	A large triangular deep-seated dark blotch above anus	Several irregular superficial black spots above origin of anal fin
Gonopodium:		
Groove	Sinistral	Dextral
Length	42 to 56	58 to 63
Terminal swelling	None	Present, small
Ray 4p	Serrae on right half only	Serrae on both halves
Dorsal origin to snout tip:		
Males	50 to 57	56 to 61
Females	54 to 60	61 to 64
Dorsal origin to caudal base:		
Males	50 to 57	46 to 51
Females	45 to 53	42 to 46
Dorsal fin, depressed length:		
Males	39 to 47 (*pittieri*) 30 to 39 (*amates*)	33 to 38
Females	35 to 37 (*pittieri*) 27 to 31 (*amates*)	29 to 33
Pelvic fin length:		
Males	19 to 25	19 to 22
Females	20 to 24	16 to 18

males of the other; no such behavior was ever observed. In aquaria *amates* seems to be a more adaptable species than *fairweatheri* with regard to temperature and water chemistry. *P. fairweatheri* reproduced readily when first brought into the laboratory but the interval between broods gradually increased until after two years no further young were delivered. Some apparently healthy adults, though only a year old, became sluggish and died. Under the same conditions of aquaria, temperature, light and feeding, young male and female *amates* became sexually mature at three to four mouths, whereas those of *fairweatheri* required eight months or longer. We may infer that there are important physiological differences between these species.

Phallichthys amates (Miller)

Diagnosis.—A robust, moderately deep-bodied, small-headed species of *Phallichthys* without severely angular contours. Juveniles with a series of 2 to 6 narrow, vertical dusky bars along the side and flank, these well developed and more numerous, about 8 to 12, in adult males; poorly defined or absent in adult females. Adult females with a large, triangular black patch on either side above genital opening. Subocular dark bar well defined, extending more downward than backward across cheek. Tissue of lower lip not developed into a fleshy pad. Dorsal fin rounded, with a black margin; anal fin of female angulate, dusted with melanophores but with the margin pale. Gonopodium sinistrally folded, long and tapering but usually not extending beyond vertical from caudal base; without distinctive terminal swelling; with a single row of unpaired serrae on right half of ray 4p; dorsal margin of subdistal segments of ray 3 with minute

oblong or T-shaped denticles; left half of ray 3 with series of broad, flat spines, strongly incurved at tips only and tapering gradually to the 5 to 10 minute terminal elements; right half of ray 3 without definite spinous processes. Adult males with posterior two anal rays and associated membranes black. Gonopodial suspensorium with heavy, blunt uncini, their tips arched downward, emerging near tips of gonapophyses II and III; gonapophysis I without uncini or other bony processes. Teeth of innner series in narrow, gently curved bands that do not have lateral, posterior projections. Vertebrae usually 29, rarely 28 or 30. Scales in lateral series 26 or 27. Dorsal rays 8 to 11, first one or two simple. Anal rays 9, rarely 10. Pectoral rays 11 to 13. Head angle 45° to 53°.

The forms *amates* and *pittieri* appear not to be separable in their gonopodial or suspensorial structures, which are highly distinctive of other species of *Phallichthys* and *Carlhubbsia*. They differ from each other chiefly in dorsal and pectoral fin ray counts, in number of simple dorsal rays (Text-fig. 6), and in length of the dorsal fin. The structural separation is apparently not complete, however, and the habitat of the forms is presumably continuous through a yet uncollected coastwise region of northern Honduras and eastern Nicaragua (Map 1). Future field work will probably narrow or close the gap between the presently known ranges. If the diagnostic characters are found to be terminal elements of clines it may be necessary ultimately to unite *amates* and *pittieri* taxonomically; if they are sympatric and remain distinct, full specific status will be indicated. Provisionally the forms are treated as subspecies.

DORSAL RAYS		PECTORAL RAYS					TOTAL
TOTAL	SIMPLE	22	23	24	25	26	
8	1	1 /	1 /	12 /	/	/	14 /
	2	/	/	1 /	/	/	1 /
9	1	1 /	2 /	94 /	2 /	3 /	102 /
	2	/	/	3 /	/	/	3 /
10	1	/	/	4 / 2	/ 2	/ 2	4 / 6
	2	/	/	/ 7	/ 4	/ 26	/ 37
11	1	/	/	/	/	/ 1	/ 1
	2	/	/	/ 4	/ 7	/ 21	/ 32
TOTAL		2 /	3 /	114 / 13	2 / 13	3 / 50	124 / 76

TEXT-FIG. 6. Frequency distribution of dorsal and pectoral fin ray counts in the subspecies of *Phallichthys amates*. In each box the number of specimens appears in the upper left for *P. a. amates*, in the lower right for *P. a. pittieri*.

Phallichthys amates pittieri (Meek)

Pl. III; Text-fig. 5

Poecilia pittieri.—Meek, 1912: 71-72 (original description; La Junta, Costa Rica).

Poeciliopsis pittieri.—Regan, 1913: 997 (La Junta, Costa Rica). Meek, 1914: 115-116 (swift rocky streams; La Junta, Parismina, Guapilis, and Virginia, Costa Rica). Henn, 1916: 120 (taxonomy; coloration). Alfaro, 1935: 237-238 (La Junta, Parismina, Río Molino, Guapilis, Costa Rica; description). Hildebrand, 1938: 309 (taxonomy; corrected distribution).

Phallichthys pittieri.—Hubbs, 1924: 10 (generic allocation; probable synonym of *P. amates*). Myers, 1925: 370 (distinct from *isthmensis* and *amates*). Hubbs, 1926: 70 (synonymy; Talamanca, Costa Rica; San San River, Almirante, Quebrada Nigua [?=Nigra], Conquantu, Western Panama). Behre, 1928: 316 (synonymy; Skoon Creek, tributary to Río Tiliri, tributaries to Almirante Bay and Chiriqui Lagoon, western Panama). Hildebrand, 1930: 6 (Siquirres, Costa Rica; characters; taxonomy). Jordan, Evermann & Clark, 1930: 190 (synonymy, in part; type locality).

Poeciliopsis isthmensis.—Regan, 1913: 997, pl. 100 (original description; Colón, Panama). Meek & Hildebrand, 1916: 325 (Colón, Panama). ?Breder, 1925: 141 (Gatun Spillway, Panama; a young female, questionable reference).

Phallichthys isthmensis.—Myers, 1925: 370 (distinct from *pittieri* and *amates*).

Material.—CNHM 7841 and UMMZ 177277 (8 yg. to ad. males and females, 19 to 50 mm. standard length), Guapilis, Limón, Costa Rica, 1912, S. E. Meek; CNHM 7839 (paratypes of *Poecilia pittieri* Meek) (14 ad. males and females, 26 to 52 mm.), Río Reventazon, La Junta, Limón, Costa Rica, April 7, 1912, Meek; UMMZ 177276 and USNM 92157 and 92158 (29 ad. males and females, 20 to 41 mm.), Siquirres, Limón, Costa Rica, Oct. 12, 1928, A Alfaro; UMMZ 72585 (7 hf.-gr. to ad. males

TABLE 7. PROPORTIONAL MEASUREMENTS OF TEN ADULT MALES OF *Phallichthys amates pittieri*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	CNHM 7839	UMMZ 177277	USNM 94108		UMMZ 177277 (CNHM 7841)	CNHM 7839	UMMZ 177276	USNM 92158		
Standard length (mm.)	34.5	33.5	27.2	27.1	26.5	26.0	24.5	23.6	22.7	21.2
Body, greatest depth	383	388	331	328	377	346	327	318	352	330
Caudal peduncle, least depth	232	230	206	221	226	231	225	212	211	203
Dorsal origin to snout tip	522	543	529	517	509	538	498	508	533	566
Anal origin to mandibular symphysis	528	555	515	517	547	515	527	530	524	557
Dorsal origin to caudal base	554	567	537	517	566	538	555	517	520	557
Anal origin to caudal base	560	585	559	568	566	558	567	542	551	495
Head length	275	278	279	284	279	288	318	284	286	311
Head width	171	161	165	170	159	165	176	191	198	203
Snout length	78	84	85	77	75	77	...	85	75	80
Orbit length	90	84	103	96	94	96	98	97	97	94
Postorbital length of head	116	107	118	125	113	115	143	140	141	127
Orbit to angle of preopercle	61	75	70	66	68	69	69	68	66	71
Interorbital, bony width	133	119	118	125	125	123	131	140	141	132
Mouth, over-all width	99	104	92	89	98	108	...	102	...	104
Dorsal fin, depressed length	464	400	393	406	442	415	396	390	396	425
Anal fin, depressed length	545	513	507	531	559	558	486	525	551	552
Caudal fin length	371	331	360	343	385	...	...	369	383	373
Pectoral fin length	252	227	243	240	272	...	265	267	247	255
Pelvic fin length	223	239	202	203	234	238	245	212	238	212

TABLE 8. PROPORTIONAL MEASUREMENTS OF TEN ADULT FEMALES OF *Phallichthys amates pittieri*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	CNHM 7839									
Standard length (mm.)	52.0	49.0	46.8	45.5	45.4	44.0	38.6	35.0	34.6	33.0
Body, greatest depth	404	422	395	398	414	409	402	391	384	379
Caudal peduncle, least depth	223	227	214	215	220	216	207	214	202	212
Dorsal origin to snout tip	560	571	551	569	564	557	583	557	552	545
Anal origin to mandibular symphysis	650	665	645	659	641	639	661	663	647	639
Dorsal origin to caudal base	498	531	515	495	504	516	490	506	512	506
Anal origin to caudal base	435	416	415	424	412	416	391	403	410	415
Head length	267	271	271	266	271	280	277	297	280	285
Head width	194	206	192	191	200	200	187	186	182	158
Snout length	85	88	88	79	84	86	86	89	87	85
Orbit length	88	90	96	97	93	91	104	103	101	106
Postorbital length of head	117	114	111	112	110	111	109	114	116	121
Orbit to angle of preopercle	71	71	68	70	68	70	67	66	66	64
Interorbital, bony width	140	149	139	143	148	145	153	149	145	142
Mouth, over-all width	102	116	107	...	108	107	117	109	116	109
Dorsal fin, depressed length	352	371	368	356	357	364	352	351	364	364
Anal fin, depressed length	252	259	252	248	256	259	259	251	260	255
Caudal fin length	346	...	...	358	...	343	365	337	...	367
Pectoral fin length	240	249	239	235	249	245	259	249	237	255
Pelvic fin length	215	229	212	215	207	223	220	217	220	233

TABLE 9. PROPORTIONAL MEASUREMENTS OF TEN ADULT MALES OF *Phallichthys amates amates*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	UMMZ 173364			UMMZ 65220	UMMZ 173280			CNHM 56168	USNM 101780	UMMZ 113403
Standard length (mm.)	29.0	28.0	28.0	25.5	24.8	22.5	20.0	19.7	16.0	14.0
Body, greatest depth	407	411	411	373	383	382	365	360	313	322
Caudal peduncle, least depth	238	236	239	235	226	227	230	218	206	215
Dorsal origin to snout tip	562	578	571	565	544	578	560	584	506	572
Anal origin to mandibular symphysis	510	525	518	506	504	507	500	497	488	479
Dorsal origin to caudal base	472	486	489	549	504	560	525	492	481	479
Anal origin to caudal base	569	582	578	596	564	578	595	584	563	565
Head length	293	311	311	302	290	302	250	315	250	286
Head width	207	214	211	196	194	191	200	193	181	193
Snout length	107	103	107	78	81	76	80	96	113	93
Orbit length	93	96	96	94	97	102	105	102	100	107
Postorbital length of head	124	132	132	137	121	129	130	122	125	107
Orbit to angle of preopercle	76	75	75	63	60	67	70	66	44	43
Interorbital, bony width	159	153	153	141	125	142	135	142	106	122
Mouth, over-all width	117	121	114	114	101	111	110	96	100	107
Dorsal fin, depressed length	348	343	386	353	363	338	375	345	300	300
Anal fin, depressed length	466	475	479	478	...	480	490	533	475	544
Caudal fin length	345	378	411	...	375	378	380	386	300	329
Pectoral fin length	272	268	289	275	278	249	250	274	244	215
Pelvic fin length	234	250	239	235	234	200	215	233	200	193

and females, 23 to 37 mm.), Skoon Creek, tributary to Río Tiliri, tributary to Río Sixaola, Talamanca, Costa Rica, Jan. 25, 1923, E. Behre and Chambers; USNM 94201 (8 ad. males and females, 28 to 39 mm.), Descampos, 1200 meters, Costa Rica, 1928, A. Alfaro; USNM 94108 (4 ad. males and females, 25 to 41 mm.), Tiribi at 1,200 meters elevation, Costa Rica, May 7, 1928, A. Alfaro; UMMZ 72587 (3 ad. females, 34 to 52 mm.), San San Creek, tributary to San San River at old San San Farm, Bocas del Toro, Panama, Feb. 5, 1923, Behre and Chambers; UMMZ 72586 (4 hf.-gr. to ad. males and females, 29 to 51 mm.), Fruitdale Creek, along railroad spur back of Almirante, July and Aug., 1921, Behre; UMMZ 72588 (one yg., 14 mm.), Quebrada Nigra, flowing into Almirante Bay, Panama, July 8, 1921, Behre; UMMZ 72584 (one ad. female, 35 mm.), Guibari Creek, tributary to Río Cricamola, below Conquantu, Panama, Feb. 23, 1923, Behre and Chambers; UMMZ 72590 (4 ad. males, 24 to 33 mm.), Nomonuen Creek, tributary to Río Cricamola above Conquantu, Feb. 22, 1923, Behre and Chambers; UMMZ 72589 (one ad. male, 24 mm.), small creek tributary to right bank of Río Cricamola below Conquantu, Feb. 26, 1923, Behre and Chambers.

Regan's (1913) record of this form from Colón was questioned by Hildebrand (1938), who stated that "Having collected rather extensively in 1911 and 1912, and again in 1935 and 1937, in the vicinity of Colón, from whence the types of *P. isthmensis* were reported, I am obliged to conclude that the species either is very rare there, or that a mistake was made in the earlier record."

Diagnosis. — A subspecies of *Phallichthys amates* with dorsal rays about equally 10 or 11, the first 2 rays (one only in 9% of specimens) simple in adult (Table 13 and Text-fig. 6). Pectoral rays 12 or 13, most often 13 (82% have 13 rays on at least one side). Dorsal fin larger than in *amates:* the depressed length 39 to 47% of standard length in adult males and 35 to 37% in adult females. Vertical bars usually present, though poorly defined, in adult females. Although our material of this subspecies averages larger than that of *P. a. amates,* the maximum sizes do not differ appreciably. The longest specimens examined are 40 mm. standard length

TABLE 10. PROPORTIONAL MEASUREMENTS OF TEN ADULT FEMALES OF *Phallichthys amates amates*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	UMMZ 173280				CNHM 56168		UMMZ 173280	CNHM 56168	UMMZ 173280	
Standard length (mm.)	37.0	32.6	31.1	29.1	27.8	27.5	26.3	25.9	25.1	25.0
Body, greatest depth	389	377	395	385	370	364	361	347	371	400
Caudal peduncle, least depth	214	209	222	210	205	207	202	201	211	228
Dorsal origin to snout tip	589	583	563	581	594	585	586	591	578	572
Anal origin to mandibular symphysis	646	641	653	632	633	647	635	656	629	644
Dorsal origin to caudal base	473	488	508	484	482	476	460	452	482	476
Anal origin to caudal base	403	411	408	409	414	393	399	386	422	424
Head length	303	307	302	299	309	309	304	313	307	296
Head width	203	205	215	206	209	211	209	216	219	208
Snout length	92	86	96	86	90	95	91	85	84	80
Orbit length	97	95	100	103	112	102	103	104	108	104
Postorbital length of head	124	132	135	127	137	135	137	131	135	140
Orbit to angle of preopercle	62	67	64	76	72	69	65	69	76	76
Interorbital, bony width	157	166	161	165	165	164	156	166	167	164
Mouth, over-all width	124	117	122	120	119	120	106	120	124	124
Dorsal fin, depressed length	297	304	293	278	281	284	278	274	...	288
Anal fin, depressed length	270	276	283	261	255	258	270	259	271	264
Caudal fin length	346	350	354	333	353	367	346	351	374	356
Pectoral fin length	257	245	264	251	259	258	247	251	239	264
Pelvic fin length	224	227	231	227	205	200	198	201	203	224

(male) and 52 mm. (female). Most adult males are between 22 and 34 mm. long.

Habitat.—Meek (1914) reported this form from swift rocky streams in Costa Rica, and specimens were collected by Alfaro at Tiribi and Descampos, Costa Rica, at altitudes of 1,200 meters; other stations are lower.

Range.—Known from the Caribbean slope of Costa Rica and western Panama (Map 1).

Phallichthys amates amates (Miller)

Pl. IV; Text-figs. 4, 5

Poecilia amates.—Miller, 1907: 108 (original description; Los Amates, Guatemala).

Poeciliopsis amates.—Henn, 1916: 120 (taxonomy; gonopodium; coloration).

Phallichthys amates.—Hubbs, 1924: 10 (taxonomy; Tela, Honduras). Myers, 1925: 370 (distinct from *pittieri* and *isthmensis*). Hubbs, 1926: 70 (synonymy; records). Jordan, Evermann & Clark, 1930: 190 (synonymy, in part; type locality). Rosen & Gordon, 1953: 24, 29, 33, 38 (mechanics of gonopodium; sexual behavior).

Material. — *Guatemala:* UMMZ 65220, CNHM 56168 (12 yg. to ad. males and females, 18 to 26 mm. standard length), Los Amates, Izabal, Jan. 17, 1905, N. Miller.

Honduras: UMMZ 173280 (25 yg. to ad. males and females, 15 to 37 mm.), Río Mapache at Masca, Cortes, April 5, 1951, Gordon and Wheeler; UMMZ 173297 (29 yg. to ad. males and females, 18 to 40 mm.), Río Tulian, Tulian, west of Puerto Cortes, Cortes, April 6, 1951, Gordon and Wheeler; USNM 101780 (one ad. male, 16 mm.), Río Chamelecón, 8 miles above San Pedro, Cortes, Jan. 19, 1936, Blanchard; UMMZ 173147 (8 yg. to ad. males and females, 12 to 24 mm.), Río Benejo, tributary to Río Chamelecón, just north of San Pedro Sula, Cortes, Mar. 18, 1951, Gordon and Chable; UMMZ 173156 (27 yg. to ad. males and females, 15 to 36 mm.), tributary to Río Ulua, Agua Priete, north of San Pedro Sula, Choloma Road, Cortes, Mar. 18, 1951, Gordon and Chable; UMMZ 56875 (one subad. male, 18 mm.), Tela, Atlantida, Mar. 14, 1923, T. H. Hubbell; UMMZ 113403 (one subad., 15 mm. and one ad. male, 16 mm.), river just outside of Tela, Atlantida, Spring, 1936, A. Greenberg; UMMZ 173177 (13 yg. to ad., 18 to 35 mm.), tributary to Río

TABLE 11. PROPORTIONAL MEASUREMENTS OF TEN ADULT MALES OF *Phallichthys fairweatheri*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	UMMZ 144186	Holotype UMMZ 172456	UMMZ 144186							
Standard length (mm.)	30.1	29.7	28.7	27.4	25.5	25.5	25.5	25.5	24.0	23.0
Body, greatest depth	415	438	411	394	392	412	361	404	354	348
Caudal peduncle, least depth	249	253	230	237	235	235	231	235	233	217
Dorsal origin to snout tip	598	606	610	584	588	588	588	596	567	565
Anal origin to mandibular symphysis	498	525	537	511	510	518	510	537	504	522
Dorsal origin to caudal base	498	495	495	493	482	506	471	471	496	461
Anal origin to caudal base	588	593	606	599	569	588	580	584	558	565
Head length	299	303	303	310	314	314	302	302	313	313
Head width	179	162	174	153	173	176	173	173	167	174
Snout length	86	88	91	88	86	90	90	90	83	87
Orbit length	106	101	105	99	110	106	110	114	104	109
Postorbital length of head	123	114	122	117	122	122	118	118	125	126
Orbit to angle of preopercle	63	61	63	66	67	71	71	63	63	65
Interorbital, bony width	116	121	122	110	110	122	114	118	125	130
Mouth, over-all width	100	98	91	91	94	94	94	98	88	87
Dorsal fin, depressed length	342	374	341	350	353	349	357	333	354	330
Anal fin, depressed length	585	606	610	602	580	596	608	624	617	622
Caudal fin length	365	380	366	365	392	373	373	377	371	369
Pectoral fin length	239	253	230	230	239	247	...	...	254	261
Pelvic fin length	219	202	195	201	212	196	196	212	204	196

Lancetilla, 1 mile south of Tela, Atlantida, Mar. 20, 1951, Gordon and Chable; UMMZ 173193 (1 ad. female, 29 mm.), Río Lancetilla at Lancetilla near Labor Camp swimming pool, near Tela, Atlantida, Mar. 22, 1951, Gordon and K. J. Davidson; UMMZ 173206 (20 yg. to ad., 15 to 39 mm.) and UMMZ 173214 (24 hf.-gr. to ad. males and females, 14 to 36 mm.), Lily Pond, Lancetilla Experimental Station, Lancetilla, near Tela, Atlantida, Mar. 22, 1951, Gordon and Davidson; UMMZ 173231 (246 yg. to ad., 15 to 37 mm.), drainage ditch on Sec. 8, African Oil Palm Plantation, San Alejo, Atlantida, Mar. 24, 1951, Gordon and Davidson; UMMZ 173221 (26 yg. to ad., 14 to 47 mm.), tributary to Río San Alejo, San Alejo, Atlantida, Mar. 24, 1951, Gordon and Davidson; UMMZ 173344 (35 yg. to ad., 14 to 30 mm.), near San Juan Benque, 48.5 km. west of La Ceiba, Atlantida, April 11, 1951, Gordon, Chable and George; UMMZ 173356 (6 hf.-gr. to ad., 20 to 27 mm.), near San Juan Benque, 47.3 km. west of La Ceiba, Atlantida, April 11, 1951, Gordon, Chable and George; UMMZ 173364 (63 yg. to ad., 19 to 39 mm.), Río Cuero, near La Masica, Atlantida, April 11, 1951, Gordon, Chable and George; UMMZ 173372 (29 yg. to ad., 18 to 37 mm.), Río Salado canal, Atlantida, April 11, 1951, Gordon, Chable and George; UMMZ 173318 (20 yg. to ad., 17 to 56 mm.), stream 6.6 km. east of La Ceiba, at Standard Fruit Company, Atlantida, April 10, 1951, Gordon and Chable; UMMZ 173329 (149 yg. to ad., 11 to 43 mm.), 18.3 km. E. of La Ceiba, Atlantida, April 10, 1951, Gordon and Chable.

Diagnosis.—A subspecies of *Phallichthys amates* with dorsal rays 8 to 10, usually 9 (10 in 4% of specimens), the first ray (2 rays in 3% of specimens) simple in adult (Table 13 and Text-fig. 6). Pectoral rays 11 to 13, usually 12 (only 4% have 13 rays on one or both sides). Dorsal fin smaller than in *pittieri;* the depressed length 30 to 39% of standard length in adult males and 27 to 30% in adult females. Vertical bars not evident in adult females. The longest specimens examined are 30.6 mm. standard length (male) and 56 mm. (female). Most males are between 20 and 30 mm. long.

Habitat.—As judged from the field records of Myron Gordon during 1951 in Honduras, this

TABLE 12. PROPORTIONAL MEASUREMENTS OF TEN ADULT FEMALES OF *Phallichthys fairweatheri*
Proportions are expressed in thousandths of the standard length.
See text for source of specimens.

Measurement	UMMZ 144186	Allo-type UMMZ 172457	UMMZ 144186							
Standard length (mm.)..	38.3	33.3	33.0	31.8	31.2	31.0	29.5	28.6	28.5	27.1
Body, greatest depth....	381	408	382	377	394	406	390	388	379	387
Caudal peduncle, least depth	225	216	221	204	218	210	220	220	218	214
Dorsal origin to snout tip	632	616	639	613	615	629	631	626	628	635
Anal origin to mandibular symphysis	674	666	651	670	683	690	671	682	667	668
Dorsal origin to caudal base	423	453	445	425	445	442	447	437	432	432
Anal origin to caudal base	418	423	406	396	417	400	417	395	421	410
Head length	313	324	324	321	330	332	325	332	337	336
Head width	183	189	191	186	192	203	193	192	193	199
Snout length	94	99	91	94	96	100	95	94	102	100
Orbit length	110	111	109	113	109	116	112	112	116	122
Postorbital length of head	133	132	133	126	128	139	136	140	133	140
Orbit to angle of preopercle	57	66	61	63	64	65	68	66	63	63
Interorbital, bony width..	151	159	152	154	154	161	159	161	158	162
Mouth, over-all width...	99	105	106	101	103	106	102	112	116	103
Dorsal fin, depressed length	300	309	303	305	304	294	322	294	309	295
Anal fin, depressed length	264	279	267	252	...	281	...	269	274	273
Caudal fin length.....	352	360	361	368	...	361	366	367	375	365
Pectoral fin length.....	...	243	239	...	250	239	254	252	246	244
Pelvic fin length.....	172	171	173	179	173	174	170	168	175	166

TABLE 13. FREQUENCY DISTRIBUTION OF DORSAL FIN RAYS IN *Carlhubbsia* AND *Phallichthys*

Species or Subspecies	Total Dorsal Rays				Simple Dorsal Rays	
	8	9	10	11	1	2
Carlhubbsia stuarti	5	116	2		1	19
Carlhubbsia kidderi	1	19				20
Phallichthys amates pittieri			45	37	7	72
Phallichthys amates amates	16	108	5		124	4
Phallichthys fairweatheri		31	11		39	2

form has rather broad tolerance to amount of current and type of bottom. At stations where moderate to large samples were taken, the current was recorded variously as none, slight, swift and rapid; the bottom as mud, mud and sand, hardpan and rubble; the water as clear, brown, stagnant and brown, and cloudy; vegetation (representing several species) as wanting or present; temperatures varied from 22° to 28° C. All specimens were seined from shallow water, usually less than three feet deep.

Range. — Known from the Atlantic coastal lowland of the Motagua River system, eastern Guatemala, east to near La Ceiba, north-central Honduras (Map 1).

***Phallichthys fairweatheri*, n. sp.**

Pls. V, VI; Text-figs. 2, 4, 5

Dextripenis evides (*nomen nudum*).—Turner, 1940: 89 (superfetation). Scrimshaw, 1944: 182 (superfetation). Scrimshaw, 1945: 234-241 (embryonic development). Scrimshaw, 1946: 21-22 (unnamed form from Guatemala; egg size).

Material.—Holotype (UMMZ 172456), an adult male, 29.7 mm. in standard length, collected in Río San Pedro de Mártir, or a branch, about ¼ mile below Laguna de Yalác, some 6 leagues (by river) upstream (east) from El Paso de los Caballos, in the Usumacinta River

basin, El Petén, Guatemala, on March 18, 1935, by Carl L. Hubbs and Henry van der Schalie. The allotype (UMMZ 172457), an adult female, 33.3 mm. long, and 6 additional half-grown to adult specimens (UMMZ 144191, 21 to 42 mm. long, were taken with the holotype (see Map 2). Additional specimens are as follows:

Río San Pedro de Mártir drainage (El Petén, Guatemala): UMMZ 144190 (19 hf.-gr. to ad. males and females, 13 to 44 mm.), Laguna de Yalác, in course of Río San Pedro de Mártir about 6 leagues by river (east) above El Paso de los Caballos, in front of old chicle station, Mar. 16, 1935, Hubbs and van der Schalie; UMMZ 144189 (76 hf.-gr. to ad. males and females, 19 to 42 mm.), Laguna de Yalác, both sides of old chicle station, Mar. 16-17, 1935, Hubbs and van der Schalie; UMMZ 144188 (one hf.-gr. and one female, 15 and 35 mm.), lagoon-like arm of Río San Pedro de Mártir at El Paso de los Caballos, March 10-14, 1935, Hubbs and van der Schalie; UMMZ 144186 (792 yg. to ad. males and females, 12 to 40 mm.), Río San Pedro, at or opposite Desempeño, just below El Paso de los Caballos, Mar. 12, 1935, Hubbs.

Río de la Pasión drainage (El Petén, Guatemala): UMMZ 144185 (9 hf.-gr. to subad., 16 to 19 mm.), Laguna de Eckibix, in savanna region southeast of Santa Ana on south shore about ¼ mile from west end, Feb. 26, 1935, Hubbs and van der Schalie; UMMZ 144187, (3 hf.-gr. to ad. females, 11 to 19 mm.), Laguna de Eckibix, extreme west end, Feb. 26, 1935, Hubbs; UMMZ 144193 (44 hf.-gr. to ad. males and females, 15 to 39 mm.), Arroyo Subín, at Trinidad, about 2 miles east of Santa Teresa, April 2, 1935, Hubbs and van der Schalie; UMMZ 144197, 144194, and 144192 (77 yg. to ad. males and females, 11 to 37 mm.), Arroyo Subín at Santa Teresa, 13 miles south of La Libertád, April 2-3, 1935, Hubbs, van der Schalie and Taintor; UMMZ 144195 (70 yg. to ad. males and females, 12 to 32 mm.), Arroyo Subín, at second rapids (about 2 miles) above mouth into Río de la Pasión, April 25, 1935, Hubbs; UMMZ 144196 (7 hf.-gr. to ad. males and females, 19 to 36 mm.), Arroyo Subín, in small bay connected with stream beside third rapids (about 2½ miles) from mouth into Río de la Pasión, April 25, 1935, Hubbs.

New River drainage (British Honduras): NYZS-GAF 5 (49 yg. to ad. males and females, 11 to 30 mm.), Hill Bank opposite campsite, inlet to lagoon of New River, Mar. 16, 1954, Gordon, Williams and Hamilton.

Río Hondo drainage (British Honduras): NYZS-GAF 6 (71 yg. to ad. males and females, 12 to 32 mm.), lagoon and creek on east bank of east branch of Río Hondo, opposite San Antonio, Orange Walk, Mar. 20, 1954, Gordon, Fairweather and Chaveria; NYZS-GAF 7 (123 yg. to ad. males and females, 11 to 31 mm.), lagoon opposite San Antonio, connected with creek to east branch of Río Hondo, Orange Walk, Mar. 21, 1954, Gordon and Chaveria.

Table 14. Frequency Distribution of Anal Fin Rays in the Forms of *Carlhubbsia* and *Phallichthys*

Species or Subspecies	Anal Rays		
	9	10	11
Carlhubbsia stuarti		19	1
Carlhubbsia kidderi	1	18	1
Phallichthys amates pittieri	79		
Phallichthys amates amates	25	2	
Phallichthys fairweatheri		22	

Diagnosis.—A deep-bodied, large-headed species of *Phallichthys* with severely angular contours. There is a series of 2 to 4 broad dusky bars on the caudal peduncle that may fuse ventrally to form a conspicuous postanal blotch, especially pronounced in adult males. In both sexes, from 6 to 7 rows of bright orange dots extend along scale rows from opercle to caudal base.[3]

Adult females with several small and irregular black spots above genital opening. Usually with an indistinct subocular dark bar that extends more backward than downward across cheek. Tissue of lower lip developed into a fleshy pad. Dorsal fin rounded, with a black margin, the middle rays longest. Anal fin of female angulate, with conspicuous dark border. Gonopodium dextrally folded, long and tapering, exceeding vertical from caudal base, with a minute terminal swelling of tough membranous tissue; ray 4p with a single row of large, retrorse proximal serrae and smaller erect terminal serrae on left half and 5 or 6 minute serrae on right half; dorsal margin of ray 3 without denticles; right half of ray 3 with series of broad, flat spines, not much incurved and tapering abruptly to the 8 or 9 slender terminal segments; left half of ray 3 without definite spinous processes. Adult males with tips of posterior anal rays black. Gonopodial suspensorium with recurved uncini, when present, developed near base of gonapophysis I and heavy subtriangular uncini near

[3] Red and yellow pigments are water soluble and for this reason are not observed in preserved specimens. The color description is taken from living and recently fixed animals from British Honduras.

Table 15. Frequency Distribution of Pectoral Fin Rays in the Forms of *Carlhubbsia* and *Phallichthys*
The counts from left and right fins for each specimen are summed

Species or Subspecies	Pectoral Rays									
	18	19	20	21	22	23	24	25	26	27
Carlhubbsia stuarti									19	1
Carlhubbsia kidderi	3	..	13	2						
Phallichthys amates pittieri							14	13	51	
Phallichthys amates amates					3	3	115	2	3	
Phallichthys fairweatheri			5	4	10	..	1			

Table 16. Frequency Distribution of Scales in Lateral Series in the Forms of *Carlhubbsia* and *Phallichthys*

Species or Subspecies	Lateral Scales					
	22	23	24	25	26	27
Carlhubbsia stuarti				11	9	
Carlhubbsia kidderi					6	14
Phallichthys amates pittieri					13	62
Phallichthys amates amates					28	6
Phallichthys fairweatheri	5	13	4			

Table 17. Frequency Distribution of Circumferential Scales in the Forms of *Carlhubbsia* and *Phallichthys*

Species or Subspecies	Scale Rows Around Body					
	21	22	23	24	25	26
Carlhubbsia stuarti			2	4	10	4
Carlhubbsia kidderi		9	8	3		
Phallichthys amates pittieri		13	7			
Phallichthys amates amates	1	9	9	1		
Phallichthys fairweatheri	1	10	9	2		

tip of gonapophysis II; uncini near tip of gonapophysis III, when present, short, heavy and sharply pointed. Teeth of inner series in broad, strongly curved bands that have well-developed, lateral, posterior projections. Vertebrae usually 28, infrequently 27 or 29. Scales in lateral series 22 to 24. Dorsal fin rays 9 or 10, usually 9, typically with only the first ray simple, rarely with 2 simple rays. Anal fin rays 10. Pectoral fin rays 10 or 11, rarely 12. Head angle 41° to 49°.

For the distinctive features of body and fin form, pigmentation and skeletal morphology, see also Tables 6, 11-18.

General Description.—A deep-bodied, robust species with high, severely angular contours. In adult males the predorsal profile is flat and rises sharply to the dorsal origin; the dorsal and ventral margins of the caudal peduncle are straight and taper rather abruptly to the caudal base. The head angle is 41° to 49°. In adult females the predorsal profile rises less sharply to the dorsal origin; the dorsal and ventral margins of the caudal peduncle slope gently toward the plane of the body axis and taper only gradually toward the caudal base. The head angle is 41° to 46°. In both sexes the snout is distinctly pointed and long, and the head deep and triangular in profile. The sharply-pointed appearance of the snout in profile view is due, in part, to the development along the outer margin of the lower jaw of a variously developed fleshy pad. In general the male is relatively deeper than the female and has conspicuously rhombic contours; those of the female are more curvilinear.

The median elevated fins in adult females originate slightly closer to the base of the caudal peduncle than to the tip of the snout and are

nearly opposite. In adult males the anal fin or gonopodium originates slightly in advance of the more posteriorly inserted dorsal fin and this is essentially the median position with reference to the body axis. The dorsal fin in both sexes is broadly rounded; the middle and posterior rays exceed the anterior ones in the depressed fin. In males the fin is more elevated and may extend almost to the caudal base. In females the anal fin has an acute anterior angle, the free margin somewhat falcate, and the third and fourth rays are much the longest. The tip of the fully developed gonopodium of the adult male extends backward to slightly beyond the caudal base. The caudal fin in both sexes is broad, subtruncate, only slightly rounded at its upper and lower posterior margin. There are 15 or 16 principal caudal rays. The rounded pelvic fins in adult females are small, about one-half the length of the anal fin; they originate approximately one-third the distance from the anal origin to the lower edge of the subopercle. In adult males pelvic rays 2 and 3 are produced; they originate just anterior to the origin of the gonopodium. The pectoral fins are broadly spatulate in both sexes and originate well below the midlateral line just behind the opercular margin, extending backward to a vertical from the dorsal origin.

The gillrakers on the outer face of the first arch, though well developed, are short and slender; they number 20 to 25.

There are three principal pigment patterns on the trunk and caudal peduncle. Two are produced by melanophores: a reticular pattern that is usually most evident above the midlateral line, and a series of vertical bars that is more pronounced in the adult male. The bars are more or less uniform and are restricted largely to the caudal region, only one or two bars of a series of 6 or more being situated anterior to the vertical from the anal origin. Two or three of the posterior bars are darker at their upper and lower extremities than midlaterally, and may ring completely the caudal peduncle. Ventrally the bars, particularly in adult males, may be so intensely black and broad that they fuse to form a large, postanal blotch. Lateral striping consists of 6 to 7 rows of bright orange dots that extend along the scale rows from the opercle to the caudal base. Adult females have three or more irregular but intensely black spots just above the anal base. In adult females both elevated median fins are evenly edged with black, except for the tips of the first three or four anal rays. In adult males the dorsal fin is black-edged; the gonopodium is unmarked, except for a stippling of melanophores and erythrophores at its base, but the tiny posterior anal rays have black tips. The other fins are without distinctive color pattern, but may show a fine dusting of melanophores, xanthophores and erythrophores, especially near their bases. The lips and the interorbital region are suffused evenly by melanophores. A short and faint suborbital bar is occasionally present.

TABLE 18. FREQUENCY DISTRIBUTION OF NUMBER OF VERTEBRAE IN THE FORMS OF *Carlhubbsia* AND *Phallichthys*

Species or Subspecies	Vertebrae			
	27	28	29	30
Carlhubbsia stuarti		23		
Carlhubbsia kidderi		2	36	3
Phallichthys amates pittieri		1	53	1
Phallichthys amates amates		1	19	1
Phallichthys fairweatheri	1	36	2	

Skeletal Morphology.—The vertebral axis, consisting usually of 28 elements (Table 18), is divided approximately in half into a precaudal or trunk series and a caudal series, the division being determined by the first hemal spine. In adult females, the first hemal spine usually emerges from the 15th vertebra. In adult males, the first attached hemal spine, which becomes sexually modified to form the first gonopophysis of the gonopodial suspensorium, is on the 14th vertebra. The precaudal portion of the axis rises gently from the vertebral centrum carrying the first hemal and then flattens out again as it approaches the base of the skull. The vertebral axis takes the form of a gentle sigmoid curve, the spinal curvature being especially pronounced in large adults. A pleural rib is present on each precaudal vertebra except the first. The first rib is long and slender and is loosely articulated with the postero-distal margin of the transverse process of the second vertebra; the rib lies against the medial surface of the pectoral girdle. Near the distal end of the rib there lies an expanded, stylet-shaped bone (see p. 7), the "postcleithrum." Each successive rib is joined to a vertebra in the same manner as the first rib; they gradually diminish in size posteriorly. In adult males the last 6 or 7 ribs become sexually modified. The last three are quite small, slender and widely separated; the preceding three or four ribs arch gently forward at their tips and converge, just above and behind the pelvic girdle. Minute epipleurals are joined loosely with the postero-proximal surfaces of the pleural ribs just below or adjacent to the level of the transverse processes; they are present on all but the last two pleural ribs. Parapophyses (see p. 7)

do not occur on any of the anterior caudal vertebrae in *Phallichthys fairweatheri.*

The contribution of the vertebral axis to the gonopodial suspensorium of the adult male consists of four specialized hemal spines. The first, or ligastyle, is a long, slender rod that lies in the primary suspensory ligament. Its dorsal detached margin migrates forward during sexual differentiation and comes to lie beneath the centrum of the 10th precaudal vertebra. The next three hemal spines or gonapophyses are specialized chiefly by the addition of bony substance to their distal and posterior surfaces. The first inclines slightly forward. It is straight except for the distal fourth which is bent sharply forward to meet the dorsal edge of the primary gonactinostal complex. A single pair of well-developed curvilinear uncini is usually present; they extend backward from the base of gonapophysis I and overlap the base of gonapophysis II. The second gonapophysis is more or less vertical. The base of the spine's shaft is slender, but it widens gradually toward the tip where it merges with a pair of extremely heavy, subtriangular uncinatoid processes. The third gonapophysis is either vertical or inclines slightly backward. It is the longest of the three gonapophyses, and its uncini, when present, are quite small, subtriangular, and are always situated near the distal tip of the spine's shaft. The actinosts of the anal fin in the adult male also are specialized and are incorporated into the suspensorial system as the gonactinosts. The first is a short, heavy, blunt rod that inclines sharply forward. It articulates with fin rays 1 and 2. Gonactinosts 2, 3 and 4 are fused to form a single highly complex plate of bone, the primary gonactinostal complex, which supports fin rays 3, 4 and 5. The complex in this species is compressed antero-posteriorly. The posterior lateral wings that are produced symmetrically outward from eccentric positions along either side of the core of gonactinost 4 are scarcely developed except at the dorsal third where they flare broadly. Gonactinost 5 lies embedded in the depression formed by the lateral wings of the primary complex. Gonactinosts 6, 7, 8 and 9 are slender and rod-like; their tips flare apart. Actinost 10 of the anal fin becomes much reduced and is incorporated into gonactinost 9 as a tiny sliver of bone. The gonactinosts are firmly anchored to the vertebral axis by means of the ligastyle and the gonapophyses. The primary gonactinostal complex is attached by ligaments to the ventral margins of the ligastyle and gonapophysis I. The tip of gonapophysis II interdigitates with gonactinosts 7 and 8, where it is held in place by means of tendons and ligaments. The tip of gonapophysis III curves forward toward the posterior surface of the final gonactinost, where it, too, is attached by means of ligamentous connective tissues.

The gonopodium in the adult male consists of the produced and modified rays 3, 4 and 5 of the anal fin. Together these rays are folded to form a hemicylinder with a broad groove along the right side of the gonopodium. When held at rest, i.e., pointed caudally, ray 3 forms the ventral border, ray 5 the dorsal border and ray 4 the lateral wall of the dextral trough. The paired halves of ray 3 are segmented to the tip of the fin and are never consolidated. The broad and flat spinous processes of the subterminal 20 to 30 segments of the right half of ray 3 are only slightly dextrally incurved to form the ventral border of the open groove; they taper abruptly to 8 or 9 paired terminal segments that form a hooked ramus. Definitive spines are wanting on the left half of ray 3 and the individual, relatively unspecialized, segments are shallow and rather poorly developed. The subterminal segments of ray 4a are flattened dorso-ventrally and their long axes are greatly extended. The terminal 10 or 11 shorter segments are closely applied to the dorsal margin of the hooked distal ramus of ray 3. A loaf-shaped membranous swelling arises from the right side of the composite terminal bony ramus. The proximal segments of the right half of ray 4p form a high, knife-like ridge. Penultimately at the distal fourth of the left half of ray 4p there is a series of approximately 15 retrorse serrae that face upward and outward away from the fin's long axis; they are preceded by 4 or 5 smaller, slender and erect, terminal serrae that extend laterally at right angles to ray 5. The elements of the distal fourth of the right half of ray 4p are delicate and thread-like except where they are modified as a series of 5 or 6 minute retrorse serrae adjacent to the bases of the larger serrae on the left half of this ray. Ray 5a is bilaterally asymmetrical; the segments of the right half of the ray are wider and more flattened than those of the left half. The segments of both, however, are greatly dilated longitudinally and extend to the tip of the fin beneath the membranous swelling as slender hair-like rods. The segments of ray 5p are extremely delicate. They are joined to the outer edges of the paired members of 5a below; those of the left half are poorly developed, becoming obsolescent at the level of the minute serrae on the right half of ray 4p; the right half of ray 5p is developed as a high, laterally compressed ridge that extends distally to the level of the smaller, erect serrae at the tip of the sinistral component of 4p. The tips of all the rays become rather slender distally

and at the extreme tip of the fin they form the beginnings of a tight spiral in which the uppermost or 5th ray shows the greatest displacement from its original axis. The gradual decrease in height of all bony elements toward the tip of each ray gives to the gonopodium as a whole a distinctly acuminate profile. Other than the hooked distal rami of rays 3 and 4a there is no distinctive terminal segment.

Relationships.—This new species is referable to the genus *Phallichthys* on the basis of the structure of the gonopodium and gonopodial suspensorium (see Text-figs. 4 and 5, and discussion on pp. 29-32). It may be separated readily from the only other known species, *P. amates,* by the characters listed in the diagnoses and in Table 6. The two species are allopatric (Map 1).

Nomenclature.—Phallichthys fairweatheri was first collected by C. L. Hubbs in El Petén, Guatemala. Hubbs thought that this fish should be the type of a new genus and assigned it the unpublished manuscript name *"Dextripenis evides,"* the generic name referring, of course, to the dextrally folded gonopodium. Specimens bearing this name were made available to C. L. Turner and N. S. Scrimshaw for their investigations on the reproductive behavior of poeciliid fishes. Both men used the name or otherwise referred to this species in their studies (see synonymy, p. 24), but did not accompany it with an adequate description. Thus, *Dextripenis evides* is a *nomen nudum* and has no validity under the International Rules of Zoological Nomenclature.

This species is named in honor of the Rev. Gerald Fairweather in acknowledgment of his participation in obtaining extensive scientific collections of fishes in British Honduras.

Range.—Phallichthys fairweatheri is known to occur in three apparently separated areas in British Honduras and northern Guatemala (Map 1). The fish from the Río Hondo and New River systems, tributary to Chetumal Bay, British Honduras, probably represent a single stock since these drainages are connected by flood plains at high water, according to L. C. Stuart (personal communication). The stocks in the Río de la Pasión and Río San Pedro de Mártir systems of Guatemala are now well separated, but future collecting in the connecting waters of the Río Usumacinta system (Map 2) may close this gap.

Habitat.—In 1935, Drs. Carl L. Hubbs and Henry van der Schalie, on the Fifth Carnegie Institution-University of Michigan Expedition, took this species 13 times in El Petén. At all stations the water was quiet or stagnant, or at least some quiet water was present. The water was clear, often blackish, at most stations though dirty at a few; vegetation was commonly present and often dense. The bottom consisted of or included soft mud at all stations. The habitat is perhaps best indicated by the conditions in Río San Pedro de Mártir (UMMZ 144186), where 792 specimens were taken: water rather dirty; vegetation slight to thick; bottom very soft mud with much hydrogen sulphide; virtually no current; shore a recently exposed mudflat.

Assessment of Taxonomic Criteria

The species of *Carlhubbsia* and *Phallichthys* are so similar in appearance that early in this study we thought they constituted a single genus. Largely on the basis of the detailed anatomical differences in the gonopodium and gonopodial suspensorium discussed below, we conclude not only that generic separation is called for, but that two distinctive phyletic lines are involved. The common feature of an asymmetrically folded gonopodium is believed to be independently evolved in these lines.

Gonopodium

The gonopodia of *Carlhubbsia* and *Phallichthys* are permanently folded to one side (sinistrally in *P. amates,* dextrally in other species), and show little internal symmetry. Although the gonopodia are superficially similar because of asymmetry, for a phylogenetic study it is essential to obtain more detailed information on individual gonopodial structures. To this end, special attention is directed to form and frequency of specialized terminal features. Some of the salient distinctions between the gonopodia of these genera are set forth in Table 19 (see also Text-figs. 3 and 4). More extended descriptions and discussions of gonopodial characteristics appear in the systematic accounts of genera and species.

In both genera the elements in ray 3 reflect the over-all symmetry of the fin, as do all the bony elements of the gonopodium. Segments which abut directly onto the permanently developed groove along one side of the fin, and which are functional components of this groove, are generally better developed than segments which arise on the side away from the direction of folding. Thus, in the sinistral *Phallichthys amates,* the dextral spinous segments near the tip of ray 3 are short with miniscule ventral processes, whereas the sinistral elements are long and curved, the ventral processes folding inward to form the ventral margin of the gonopodial groove. In *P. fairweatheri* and the species of

Carlhubbsia the spines are present only on the right half of the ray and face into the gonopodial groove, again to form the ventral margin of the partially closed channel. An exception to this generalization involves those specialized holdfast structures that are believed to assist in copulation; for example, the distal serrae on ray 4p in all four species face outward, that is, away from the gonopodial groove on the convex surface of the permanently folded fin.

In *Phallichthys* the gonopodium is comparatively simple, having few specialized terminal features; there are unilateral distal spines on ray 3 and serrae on ray 4p, the subterminal elements of all rays are uniformly simple and the fifth ray is unspecialized (Table 19). In *Carlhubbsia,* by comparison, the gonopodium is complex. Each ray with the exception of the anterior branch of 5 is terminated by a series of specialized elements: in addition to spines on ray 3 and distal serrae on ray 4p there is a small terminal hook on ray 3, a series of laterally dilated terminal segments on ray 4a, a disrupted ray 4p and posterior serrae on ray 5p.

Gonopodial Suspensorium

In poeciliid fishes the development of such suspensorial structures as the ligastyle and the gonapophyses is influenced by over-all growth patterns and time and rate of sexual differentiation (Rosen, ms.). Growth and maturation, in turn, are affected by a variety of environmental factors, such as nutrition, light and temperature. But underlying genetic patterns appear largely to control the expression of some structures irrespective of body size or form; if these can be properly identified they provide indications of natural relationship. The position of uncini on the gonapophyses and their form, as well as the shape of the primary gonactinostal complex, have always proved to be relatively constant within a related group of species.

The form of the ligastyle and the orientation of the gonapophyses are apparently controlled in large part by the form of the body. In *Carlhubbsia kidderi,* the slenderest of the species under consideration, the ligastyle is reduced to a rudiment of bone embedded in the primary suspensory ligament and all three gonapophyses incline forward at a sharp angle. In *Phallichthys amates,* of intermediate body depth, the ligastyle is quite small but in some individuals is prolonged into a slender rod of bone equal in length to the diameter of a vertebral centrum; only the first gonapophysis is bent sharply forward. In *C. stuarti* and *P. fairweatheri,* the two deepest-bodied species, the ligastyle is a well-developed, long bony rod and the anal fin supports have a more nearly vertical orientation. In each, the angle between the gonapophyses and the vertebral column is roughly proportional to the distance between the gonactinosts and the vertebral axis (Text-fig. 5).

Positional relationships and form of the uncini on the three gonapophyses have proved extremely effective in interpreting the relationships in these fishes. As may be seen from Plate VI, Text-fig. 5, and Table 19, there is measurable individual variation within species and between species of *Carlhubbsia* and *Phallichthys* in the orientation and extent of development of uncini. But what matters from the viewpoint of mechanical control is not the precise point at which an uncinus arises *per se,* but the total adaptation for the job of suspension, which if the point is constant can be achieved by fine adjustments in size, orientation and rigidity of the uncini. In the species of *Carlhubbsia,* for example, *C. stuarti* lacks uncini on gonapophysis III, but the uncini on gonapophysis II are so long that they overlap the shaft of number III at the same point at which two tiny uncini arise on element III in *C. kidderi.* Two specimens of *C. kidderi* were found with well developed uncini on gonapophysis II, but none on III. The same broad functional problem of suspension has been solved repeatedly in different ways within the Poeciliidae as a whole. For this reason it is significant that, despite individual and specific differences, each genus presents a relatively distinctive pattern with respect to the topographic relations and basic morphology of these gonapophyseal processes.

In both *Carlhubbsia* and *Phallichthys* the relative size of the primary gonactinostal complex remains fairly constant, without reference to variations in body proportions or ultimate size attained. As a result, in the more slender species, *C. kidderi* and *P. amates,* this bony complex closely approaches the vertebral axis, whereas in the deep-bodied forms, *C. stuarti* and *P. fairweatheri,* it is well separated. As previously noted, the length of the ligastyle and the angle of inclination of the first gonapophysis compensate for these differences. Thus, the relative constancy within each genus suggests conservativeness in the gonactinostal complex, and the finding of differences between the groups of species (genera) emphasizes its reliability. In the species of *Carlhubbsia* the primary complex is greatly dilated along the antero-posterior dimension; in the species of *Phallichthys* it is narrow (Text-fig. 5). This difference is brought about in three ways: when incorporated into the complex acti-

TABLE 19. SIGNIFICANT CONTRASTING CHARACTERS OF *Carlhubbsia* AND *Phallichthys*

Character	*Carlhubbsia*	*Phallichthys*
Gonopodium (adult male):		
Ray 3	15 to 20 subterminal segments with flattened incurved spinous processes; 5 to 8 distal elements simple; with small curved terminal hook	20 to 30 subterminal segments with flattened incurved spinous processes; 8 or 9 distal elements simple, forming a ventrally hooked ramus; no terminal hook
Ray 4a	Approximately 15 distal segments much elevated; not forming a hooked ramus	5 terminal segments compose a minute hooked ramus that arches downward over the hooked tip of ray 3
Ray 4p	Very thin distally, closely joined to ray 4a; obsolete from segments 20 to 25 of ray 4a (counting apico-basally) to about segment 15 (ray 4a) where it reappears as a clustered series of 4 to 8 unpaired retrorse serrae	Terminal series of up to 17 or 18 well-developed, retrorse serrae
Ray 5	Ray 5p with sinistral, retrorse serrae on 6 or 7 (*kidderi*) or about 15 (*stuarti*) terminal segments; serrae fused with distal elements of 5a and separated from unspecialized segments of 5p (*kidderi*) or distinct and continuous with unspecialized segments (*stuarti*)	Little specialized except in symmetry; without serrae
Gonapophyses (adult male):		
Uncini on I	Slender and straight, tilted downward, of moderate length (*kidderi*) or long (*stuarti*); arising near base of gonapophysis	Slender and short, curved upward, arising near base of gonapophysis (*fairweatheri*) or absent (*amates*)
Uncini on II	As on gonapophysis I but longer and stronger, situated slightly farther down gonapophysis but in same plane as uncini on I	Stout, broad based, short, curved downward, arising on distal half of gonapophysis; not in same plane as uncini on I
Uncini on III	Absent (*stuarti*, rarely in *kidderi*) or rather short, moderately broad and straight, arising near middle of gonapophysis in same plane as uncini on I and II (*kidderi*)	Short and very broad with tips obtuse, frequently curved downward, arising in distal third of gonapophysis, not in same plane as uncini on I and II (usually absent in *fairweatheri*)
Primary gonactinostal complex (adult male):	Broad; antero-posterior breadth about 1/3 to 2/5 length	Narrow; antero-posterior breadth about 1/4 length
Preorbital bone	More sculptured; with well-developed process projecting backward	Simple; roughly subtriangular in outline
Dorsal fin	More or less angulate; the free edge falcate	Rounded

nosts 2, 3 and 4 are distinctly separated in *Carlhubbsia*, in close apposition in *Phallichthys;* the anterior plate of bone on gonactinost 2 is broadly dilated or distended anteriorly in *Carlhubbsia*, only narrowly so in *Phallichthys;* and the posterior lateral wings on gonactinost 4 are accentuated in *Carlhubbsia*, only moderately developed in *Phallichthys*.

In summary, there are three principal differences between the suspensoria of *Carlhubbsia* and *Phallichthys*. In *Carlhubbsia* the uncini always originate along the proximal half of the shaft of a gonapophysis; in *Phallichthys* the uncini of gonapophysis I originate on the proximal half, but on gonapophyses II and III they originate on the distal half of the spine's shaft.

In *Carlhubbsia* the uncini are long, straight, and they merge with the gonapophyses rather abruptly; in *Phallichthys* the uncini are short, they are curvilinear, and they merge with the gonapophyses gradually, causing the spines to appear swollen at their tips. In *Carlhubbsia* the primary gonactinostal complex is greatly dilated anteroposteriorly; in *Phallichthys* it is relatively narrow.

Head Skeleton and Dentition

In both *Phallichthys* and *Carlhubbsia* the head skeleton is typical of such poeciliid species as *Poecilia vivipara* and *Xiphophorus maculatus* whose principal diet consists of organic debris, minute aquatic organisms and plant material. In both genera, the base of the cranium is high and firmly fixed in position by means of well-developed supraoccipital and epiotic processes, which join the high, expanded neural crests of the cervical vertebrae by means of strong ligaments. This type of deep, immobile skull is characteristic of other sluggish, forage-feeding fishes as well. Similarities in basic skull form in the two genera may as reasonably be interpreted to reflect similar feeding behavior as intimacy of relationship. The only superficially obvious skull differences occur in the orbital bones. In *Carlhubbsia* the preorbital (lacrymal) is more sculptured and has a well developed process produced backward toward the lateral ethmoid; in *Phallichthys* it is simple, roughly subtriangular in outline.

The dentigerous features of premaxillae and dentaries are similar in *Carlhubbsia* and in *Phallichthys,* although the shape of the inner bands is distinctive in *P. fairweatheri.* Such nutritional adaptations as tooth structure and size and orientation of dentigerous borders are notably variable in some poeciliid genera (e.g., *Poeciliopsis*), and the taxonomic usefulness of these characters is limited accordingly. To some degree, tooth structure varies independently of basic architecture of mouth parts. In *Carlhubbsia* and *Phallichthys,* for example, the jaws are weak, blunt and loosely joined at the midline. Yet *Phallichthys fairweatheri* has a broad, curved inner band of minute teeth and *P. amates* and the species of *Carlhubbsia* have a nearly straight, narrow inner band of longer teeth. Jaw structure is fundamentally similar in the poeciliid genera *Aulophallus, Quintana* and *Girardinus.* In *Aulophallus* the teeth of the outer series are setiform, in *Quintana* they are more or less conical and sharply pointed, and in *Girardinus* they are broadly oblanceolate. In general, it appears that in this family dental characters offer little hope of aiding phylogenetic analysis. Systematic arrangements of the Poeciliidae and other cyprinodontiform groups based on these and other nutritional features have been subjected to just criticism (e.g., Hubbs & Turner, 1939; Miller, 1956).

Sensory Canals

Both *Carlhubbsia* and *Phallichthys* exhibit moderately well developed sensory canal systems, although problems in their direct comparison arise due to changes in the canals associated with size and age. Closed canals of large females may be represented only as open grooves in juveniles, in small adults, or even in mature males. Thus, of ten adult females of *Carlhubbsia kidderi* (UMMZ 144203) 28 to 34.5 mm. in standard length, the supraorbital canal is closed between pores 2 to 4 and 6 and 7 in nine, and from 2 to 3 and 6 to 7 in one, the smallest; the preopercular canal is closed and has 7 pores in eight, has 8 pores in one, and has 6 pores and a short groove in the smallest; and the preorbital canal has 3 pores in all. But of ten adult males from 16 to 20.5 mm. long from the same series, the supraorbital canal is developed from 2 to 4 and from 6 to 7 in six, from 2 to 3 and 6 to 7 in one, from 6 to 7 in one, and is represented only by grooves in two; the preopercular canal consists of 6 pores in seven, of 4 in two, and of 3 in one, the remaining portions being evidenced by open grooves; and the preorbital canal has 2 pores and a groove in three and consists of a groove only in seven. In *Carlhubbsia* and *Phallichthys* the mandibular canal is never developed, in the definitive condition the preopercular canal has 7 pores in all species, and the preorbital canal has 3 pores except for *C. stuarti* which has 4. The apparently definitive condition for the supraorbital canal is to have a tube with three pores above the eye (pores 2, 3 and 4a) and one with 2 behind it (pores 6 and 7) in all species. This arrangement is usually found in both species of *Carlhubbsia,* at least in adult females, and sometimes, especially in very large females, in the forms of *Phallichthys.* In most adults of *Phallichthys,* however, only the short postorbital section of the canal is covered. In three of ten adult females of *C. stuarti* examined, there is a third remnant of the supraorbital canal connecting pores 4b and 5.

In general, *Carlhubbsia* and *Phallichthys* are similar in the pattern of their sensory canals. But this pattern is essentially the same as that reported by Gosline (1949) for *Mollienesia latipinna* and *Girardinus metallicus* and is close to that found in *Platypoecilus (= Xiphophorus) maculatus* and *Poeciliopsis* sp. We therefore find it impossible at this time to treat the simi-

larities in sensory canal pattern in *Carlhubbsia* and *Phallichthys* as indicative of a phylogenetic relationship.

Relationships of *Phallichthys* and Status of the Poeciliopsinae

Based chiefly on similar asymmetric modifications of the gonopodium of adult males, the genera *Phallichthys, Poeciliopsis, Poecilistes, Aulophallus, Xenophallus, Phalloptychus* and *Carlhubbsia* (as *Allophallus*) have been associated as a subfamily, the Poeciliopsinae, by Hubbs (1924, 1926, 1936). It has been inferentially suggested (Miller, 1955:50) that the monotypic genus *Poecilistes* is a generic synonym of *Poeciliopsis*. We find that the inner teeth in *Poecilistes pleurospilus* are much reduced in number, uniserial, and are restricted to the lateral ends of a row in each jaw. The supposed absence of the inner teeth has been employed as the primary basis for removal of *Poecilistes* from *Poeciliopsis* (Hubbs, 1936:233), a separation that can no longer be accepted. As a result of comparative morphological study we believe that the above association consists of four divisions, one comprising *Phallichthys, Poeciliopsis* and *Aulophallus*, the others *Phalloptychus, Xenophallus* and *Carlhubbsia* respectively. Each division is representative of what appears to constitute a distinctive phyletic line.

The diagnostic features of *Poeciliopsis* (see also Hubbs, 1936, and Hubbs & Miller, 1954) include: (1) body slender with posteriorly-placed dorsal fin; (2) gonopodium tightly rolled into a partially closed tube; ray 4p with paired, asymmetrical terminal retrorse serrae; terminal segments of ray 5 simple, without ornaments; rays 7 and 8 converging or in contact along middle of their lengths; (3) gonapophyses with uncini nearer distal than proximal end of shaft; uncini with broad bases and ventrally-arched, blunt tips; and (4) primary gonactinostal complex greatly dilated antero-posteriorly and deeply notched at dorsal margin, the incorporated shafts of actinosts 2, 3 and 4 flaring outward and upward away from the consolidated basal components.

With two exceptions, *Aulophallus* shares the above features with *Poeciliopsis*. It differs principally in the development of unpaired serrae on ray 4p of the gonopodium and in the transverse widening of terminal elements of ray 5a which in *Poeciliopsis* are greatly compressed. Dentition, used formerly to separate these groups, should be carefully reviewed, since within *Poeciliopsis* tooth form and orientation and size of the dental ridges are highly variable, according to Robert R. Miller (personal communication—see also Hubbs, 1936: 235). The demonstrable intimacy of relationship between *Poeciliopsis* and *Aulophallus* may eventually necessitate their merger.

That the species of *Phallichthys* may have evolved directly from a form having symmetrical or nearly symmetrical gonopodial elements rather than from a *Poeciliopsis*-like fish, is suggested by the presence of only moderately incurved spinous processes on ray 3, the presence of a high, laterally compressed ridge on 4p and the lack of definitive closure of the groove at any point along the gonopodium. In the evolution of asymmetrical genitalia in Poeciliidae, formation of a fixed unilateral groove is made possible by folding of the rays, incurving of elements of ray 3 to form the ventral wall of the groove and compaction and superimposition of rays following loss of basic rotatory mechanisms (Rosen & Gordon, 1953). The segment ridge on ray 4p of the gonopodium in *Phallichthys* almost certainly is a remnant of the dorsal center of rotation (between rays 4 and 5) that is a constant feature of almost all symmetrical gonopodia. This, in turn, suggests that in *Phallichthys* some rotatory movements may occur during fin erection; additional mechanical adjustments would seem almost obligatory in order for the ray 5 complex to be apposed with the only moderately incurved spines of ray 3. The low degree of closure of the unilateral groove of the resting fin in both *P. fairweatheri* and *P. amates* contrasts sharply with the compact, twisted or even helical gonopodia in species of *Poeciliopsis*. In the latter forms, maximal folding of rays 3, 4 and 5 occurs during development, and no further positional adjustments accompany fin erection. The morphological differences separating these genera are summarized in Table 20.

Despite many differences in the details of their gonopodia and gonopodial suspensoria, *Phallichthys* and *Poeciliopsis* display a number of broad similarities. In their gonopodia we find: (1) segments on one-half of ray 3 rolled inward to form the ventral wall of the unilateral groove; (2) segments of ray 4a simple and without ornaments; those of 4p with a series of terminal retrorse serrae (paired though asymmetrical in *Poeciliopsis* and *Phallichthys fairweatheri*, unpaired in *Phallichthys amates* and *Aulophallus*); and (3) segments of ray 5 simple and much reduced. In their gonopodial suspensoria, we find: (4) uncini usually arising well out on gonapophyseal shaft; and (5) uncini with heavy bases, their tips blunt and developed in an arch downward. In view of over-all similarities in form and orientation of their male secondary

TABLE 20. COMPARISON OF *Phallichthys* AND *Poeciliopsis**

Character	*Phallichthys*	*Poeciliopsis*
Form	Body deep and compressed	Body slender and more terete
Body depth (percent. of standard length)	32 to 44	About 22 to 31
Body circumference scales	21 to 24	19 to 21
Dorsal origin (females)	Slightly in advance of anal origin	Decidedly behind anal origin
Dorsal rays	8 to 11; infrequently 8	Usually 7 or 8
Vertebrae	27 to 30, modally 28 or 29	29 to 33, modally 30 to 32
Suborbital dark bar	Present, more or less oblique; sometimes faint	Absent
Mandibular canal	Never developed	Often present; sometimes absent
Gonopodium:		
Form	Asymmetry less extreme; no definitive closure of groove	Twisted into a tightly rolled and partially closed tube
Spinous processes on ray 3	Moderately incurved	Strongly incurved
Subterminal segments of ray 3	Not consolidated	Usually several consolidated to form a slender rod
Ray 4p	With high laterally compressed ridge; terminal retrorse serrae paired or unpaired	Without high ridge; terminal retrorse serrae paired
Rays 7 and 8	Symmetrical; well separated at middle of their lengths	Distorted; converging or in contact at middle of their lengths
Suspensorium:		
Gonapophyseal uncini	Stout, broad-based, short, curved downward, usually emerging on distal half of spine; not lying in same plane	Stout, broad-based, usually short, curved downward, emerging on distal half of spine; not lying in same plane
Gonactinostal complex	Narrow antero-posteriorly; neither dilated nor notched at dorsal end	Dorsal end dilated antero-posteriorly and deeply notched

* Dr. Robert R. Miller is engaged in a revisionary study of *Poeciliopsis* which, when completed, will permit a much more adequate contrast of these genera.

sexual specializations, it is possible that *Phallichthys* and *Poeciliopsis*, with *Aulophallus*, may have radiated from a common prototype in which developmental patterns for gonopodial asymmetry were first becoming established. The difference in direction of gonopodial asymmetry of *Phallichthys fairweatheri* (dextral) and *P. amates* (sinistral) could have come into existence by means of a genetic "switch" mechanism that controlled direction of asymmetry at a critical point during epigenesis; it does not necessarily represent a fundamental divergence between these two species.

The Uruguayan genus *Phalloptychus* is characterized by having extremely well developed, projecting and more or less vertically oriented, unpaired serrae at the tip of gonopodial ray 4p, and long, straight and slender suspensorial uncini that emerge from the bases of and extend horizontally backward from gonapophyses II and III. With reference to their zoogeography and comparative morphology, the two tiny species of this South American genus are highly distinctive. We find no basis for an alliance between this genus and the group including *Phallichthys, Poeciliopsis* and *Aulophallus.*

Similarly, the Central American *Xenophallus* has no special combination of gonopodial or suspensorial traits which, in our opinion, associate it with *Poeciliopsis* and its allies. The gonopodium (see Rosen & Gordon, 1953:29) is remarkably simple, without serrae or spines; it is specialized only in the sinistral or dextral folding and in the prolongation of the tip of ray 4a as a single, consolidated curved bony rod. In its suspensorium, uncini on gonapophyses I, II and III arise on the basal half of the gonapophyseal shafts along a single axis, and they are linear and rather slender. The relationships of both *Phalloptychus* and *Xenophallus* will be discussed more fully in a forthcoming publication (Rosen, ms.).

Evidence has been presented above suggesting that *Carlhubbsia* is not intimately related to *Phallichthys* or to any other member of the heterogeneous assemblage heretofore lumped as the subfamily Poeciliopsinae. Nowhere in the group are there other forms having suspensorial and gonopodial details like those of *Carlhubbsia*. Among the remaining major groups of poeciliids we find a constellation of features which most closely resembles those of *Carlhubbsia* in the endemic Cuban genera *Quintana* and *Girardinus* (see below, pp. 35-39).

From a functional standpoint it may seem elementary to suggest that asymmetric folding of the primary anal rays to form a permanent closed or partially closed tube would produce a highly adapted vehicle for sperm transfer. All poeciliid fishes not so equipped create a transitory tube by folding the anal rays at each copulatory attempt. Thus all members of this group, and indeed others that similarly employ the anal fin to effect internal fertilization, are in a sense preadapted to the evolution of a permanently asymmetric genitalium. Permanent folding permits structural modification to enhance the effectiveness of the mechanism. That a development so useful in the maintenance of species should evolve but once in this family in which gonopodial plasticity is abundantly demonstrated is conceivable, but a polyphyletic origin of asymmetry is certainly to be anticipated.

Justification for dismemberment of the Poeciliopsinae rests solidly with the weight of evidence from study of the fine details of the gonopodium (apart from its asymmetry) and gonopodial suspensorium. Gonopodial asymmetry, far from being a uniting character, almost certainly has appeared independently at least five times within the Poeciliidae [in *Phallichthys-Poeciliopsis-Aulophallus, Phalloptychus, Xenophallus, Carlhubbsia* and in *Xenodexia ctenolepis*, regarded by Hubbs (1950) as constituting a distinct subfamily], and in two other cyprinodontiform families, the Jenynsiidae and Anablepidae. The beginnings of such a pattern can be seen in still another poeciliid genus, *Quintana*, in which serrae on ray 4p of the gonopodium are always twisted sinistrally.[4]

[4] C. L. Hubbs (Occ. Pap. Mus. Zool., Univ. Mich., 302: 1-3, 1935), in an article entitled "Studies of the fishes of the Order Cyprinodontes. XIV. *Plectrophallus* regarded as a distinct genus," figured the gonopodium of the poeciliid *Plectrophallus tristani* (Fowler) to illustrate the asymmetric folding of the rays. This would, of course, represent yet another example of the independent origin of asymmetry in the family, since *P. tristani* is probably allied to the species of *Brachyrhaphis*. *P. tristani* is known from but one specimen, however, and we have not seen this.

Carlhubbsia and the Cuban Endemic Poeciliids

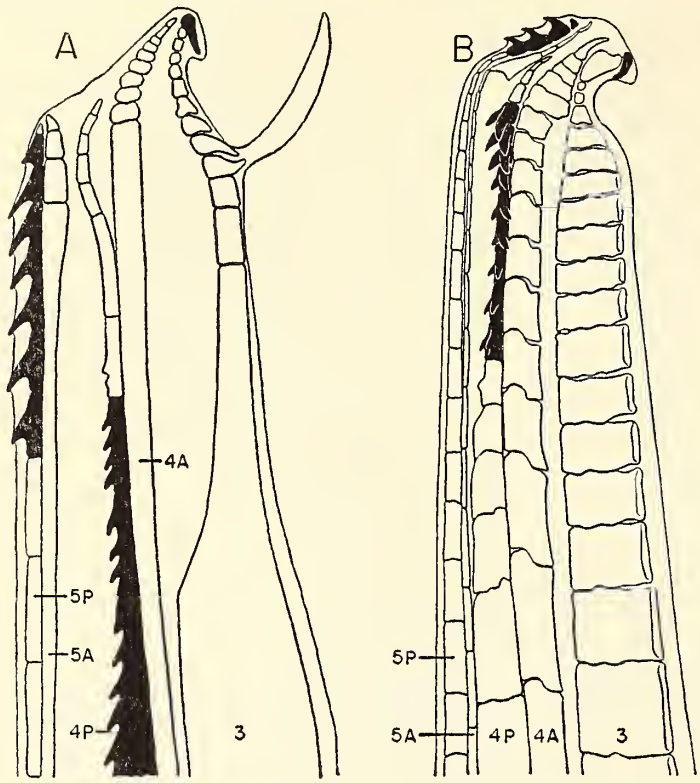
Our attempt to decipher the relationships of *Carlhubbsia* has led us to investigate the Cuban poeciliids which in current classification constitute the tribes Girardinini and Quintanini. Howell Rivero & Rivas (1944) called attention to the integrity of the girardinins, and pointed to the distinctiveness and uniformity of their gonopodia and suspensoria. Suspensorial structures were studied further by Howell Rivero (1946) and the compactness of the Girardinini was again emphasized. This group, now numbering about 10 recognized species, was arranged in five genera that were separated chiefly on the basis of dentition and mouth structure. There are at most minor differences, mostly average features, in the gonopodia of all groups except *Toxus*, which, though sharing the prominent horn-like terminal appendages and all specialized bony structures, has the most distinctive gonopodium of the five genera. Howell Rivero & Rivas (1944: 14) summarized their study of these fishes as follows:

"In their fundamental features, therefore, the genera of the Girardinini are in almost complete agreement. The radiative adaptation of these genera seems to have been related chiefly to food habits, for most of the generic characters involve the structure of the jaws, mouth and teeth (see key to genera). There is every reason to believe that the genera of the tribe have evolved in Cuba, after a single ancestral species migrated into what is now that island."

As mentioned above, dentitional characters have been seriously over-emphasized in the systematics of the cyprinodontiform fishes. The gonopodia and suspensoria of the girardinin fishes (Howell Rivero & Rivas, 1944; Howell Rivero, 1946; Rosen & Gordon, 1953: 27) show numerous and striking similarities. This is so manifestly a compact group phylogenetically and zoogeographically that we prefer to classify the species in a single genus, *Girardinus* Poey, of which *Glaridichthys* Garman (including *Glaridodon* Garman), *Toxus* Eigenmann, *Dactylophallus* Howell Rivero & Rivas and *Allodontium* Howell Rivero & Rivas are generic synonyms. Thus, *Girardinus* as amended is equivalent to the Girardinini of recent authors. Since *Quintana* is the only genus in the tribe Quintanini, these terms also are equivalent in scope.

Girardinus *and* Quintana

That *Quintana atrizona* is highly distinctive was clearly indicated by Hubbs (1934) in the original account. He placed the genus in the



TEXT-FIG. 7. Distal tips of the gonopodia of: A. *Girardinus denticulatus* (Garman), and B. *Quintana atrizona* Hubbs, as seen from the left side. Bony elements being compared are shown in solid black.

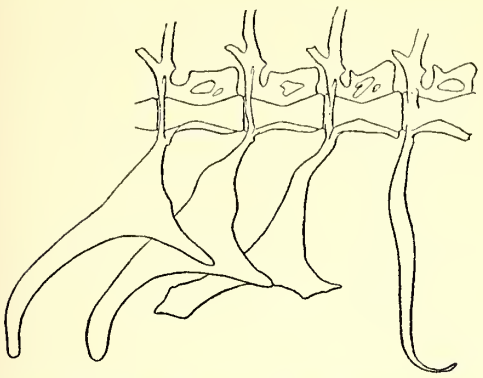
subfamily Gambusiinae and, hesitantly, in the tribe Heterandriini. Certain similarities to *Gambusia, Allogambusia* and *Girardinus* (*sensu lato*) he regarded as more plausibly due to parallelism and convergence than to common origin. Howell Rivero & Rivas (1944: 13) granted separate tribal status to *Quintana,* but commented that "Our continued studies have emphasized the integrity of the Cuban group Girardinini, although the gonopodial characters of *Quintana* (see key) somewhat confuse our views as to the isolated position of this group." Howell Rivero's description and figure of the suspensorial apparatus of *Quintana* (1946) clearly indicate the unique features. He reported that only the second of the three gonapophyses is appreciably specialized and complex, an observation not fully substantiated by our material.

Recent evidence has been found to support the hypothesis that *Quintana* and *Girardinus* arose on Cuba from invasions of a single or of two closely related forms (Rosen, ms.). If this is true the affinities of these genera should be emphasized by their close association in the systematic structure of the family. For the present purposes it is sufficient to note certain structural similarities.

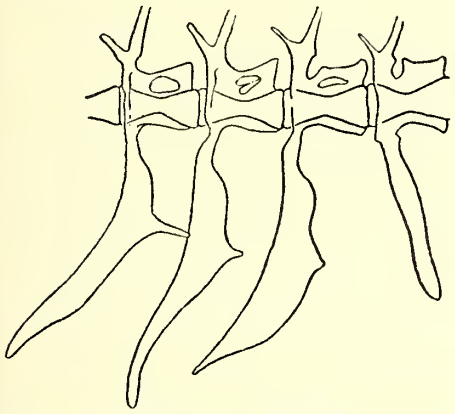
In the gonopodia and gonopodial suspensoria of *Quintana* and *Girardinus* (Text-figs. 7, 8 and 9), we find the following diagnostic structures that we interpret as homologous in these genera: (1) a minute, recurved, terminal hook on ray 3; (2) weak retrorse serrae on ray 4p; (3) moderately to well-developed serrae on ray 5p; (4) three highly specialized gonapophyses; and (5) uncini developed on all three gonapophyses. To these may be added similarities of body form and fin shape. All species are streamlined, all have the dorsal and ventral trunk profiles symmetrically arched, and in all the caudal peduncle is long and slender. The median fins, particularly the dorsal, tend to be sharply pointed and even falcate.

Characters Indicating Relationship of Carlhubbsia *with* Quintana *and* Girardinus

If we deal first with the primary terminal specializations on rays 3, 4 and 5 of the gonopodium (Text-fig. 10) and allow for the minor modifications which are probably to be attributed to asymmetric growth, we find that with but one important exception the gonopodia of *Quintana* and *Carlhubbsia* are much alike. They share the following similarities: in ray 3 both have a series of stout proximal elements that become considerably compressed apico-basally



TEXT-FIG. 8. Axial division of the gonopodial suspensorium of *Girardinus creolus* (Eigenmann), showing the three gonapophyses and a slightly modified hemal spine. Anterior to the left.



TEXT-FIG. 9. Axial division of the gonopodial suspensorium of *Quintana atrizona* Hubbs, showing the three gonapophyses and a slightly modified hemal spine. Anterior to the left.

towards the tip of the ray. At the exposed margin of the ray about ten terminal segments that are decidedly higher than long are produced into broad, flat spines; the most distal extent of the posterior spinous processes contribute to the formation of the eccentric groove that arises obliquely from the ventral margin of this ray. In *Carlhubbsia* the single, dextral eccentric groove forms the permanent anterior edge of the partially folded gonopodium. In *Quintana* the eccentric grooves on both sides of the ray serve as the anterior edge of the transitory channel when the gonopodium is swung forward and to one side during fin erection (Rosen & Gordon, 1953: 18-23). In both, ray 3 is terminated by an abruptly widened, segmented or consolidated, bony complex and by a minute recurved bony hook (in *C. stuarti,* the hook is replaced by an uncalcified though rigid membranous structure). In both *Carlhubbsia* and *Quintana* ray 4a is slender proximally and abruptly shortened and widened distally. Ray 4p in both genera is slender distally (usually obsolescent in *Carlhubbsia*) and bears a cluster of retrorse serrae. These serrae are rotated sinistrally in both genera. Ray 5p in both *Quintana* and *Carlhubbsia* is terminated by a series of erect though short serrae. The subdistal segments of ray 5a, which in *Quintana* develop as an "elbow-like" structure, and the profound asymmetry in *Carlhubbsia* are the only gonopodial features in which the two groups differ significantly.

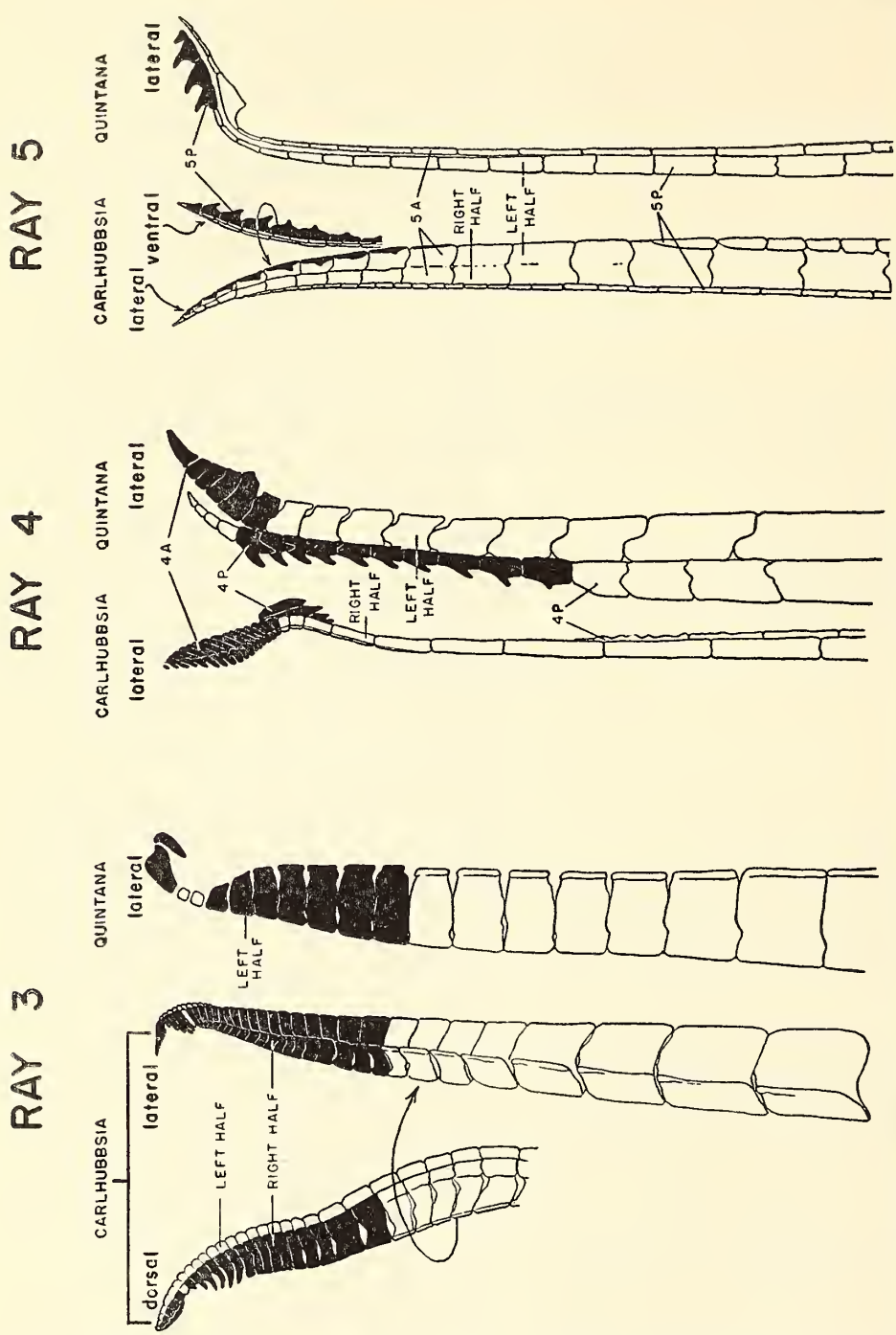
In the gonopodium of *Girardinus,* ray 4a is not expanded distally, the spines of ray 3 are smaller, less numerous, and are delicately pointed, and a conspicuous pair of fleshy subradial processes originates below the spines of ray 3 and extends in an arc forward and laterally.

Of all the taxonomically significant morphological details, those of the gonopodial suspensorium show the most striking similarities among the species of *Carlhubbsia, Quintana* and *Girardinus* (Text-figs. 5, 8 and 9). In each, the three gonapophyses all bear uncinatoid processes that lie more or less in the same plane at an angle of approximately 30° with the vertebral axis. The uncini in all are limited to the proximal portion of the spine's shaft, and are always linear, pointed and rather slender.

The gonapophyses of the suspensorium of *Quintana* were described and figured by Howell Rivero (1946) as being simple and having but a single pair of uncini. Actually the suspensorium of a paratype of *Q. atrizona* (Text-fig. 9) shows small uncini on all three gonapophyses. *Quintana* and *Girardinus* are considerably more alike than had been thought from earlier studies. In suspensorial structure they differ only in the extent of development of the uncini; in *Girardinus* (as well as in *Carlhubbsia*) the uncini are relatively large, strongly produced, and overlapping. Howell Rivero's Figure 10, which shows almost complete lack of specialization of gonapophyses I and II of *Quintana,* indicates that he may have worked with subadult males in which the fine details of suspensorial structure had not yet fully developed.

Résumé of Morphological Analysis and Conclusions

Since the similarities in certain skeletal features in *Carlhubbsia, Quintana* and *Girardinus* are not always clearly defined and the alleged homologies may seem questionable, it may be contended that many or all of the resemblances are examples of evolutionary parallelism. It is



TEXT-FIG. 10. Comparison of the disarticulated principal gonopodial rays of *Carlhubbsia kidderi* (Hubbs) and *Quintana atrizona* Hubbs. The individual rays are oriented as in the articulated gonopodium when viewed laterally. Rays 3, 4, and 5 of *Carlhubbsia* are viewed from the right, those of *Quintana* from the left, in order to reveal the maximum number of details. The compared elements are in solid black. An additional view of ray 3 of *Carlhubbsia* shows the tip of this ray in dorsal view after it has been rotated 90° to the left.The additional view of ray 5 of *Carlhubbsia* shows the tip of this ray in ventral view after it has been rotated 90° to the right. Curved arrows indicate direction of rotation necessary to bring these back to the normal (lateral) position.

often possible, however, to recognize the existence of spurious similarities that define an adaptive trend in gonopodial and suspensorial structure, and at the same time to recognize evidence for homology where it exists. Considering the totality of morphological evidence for the similarities between *Quintana* and *Girardinus*, we regard as homologous the distal serrae (ray 5p), the proximal serrae (ray 4p) and the terminal hooks (ray 3) in their gonopodia. The spines (ray 3), however, may have developmentally different origins because in *Girardinus* the spines are always intimately associated with large fleshy ventral processes, structures that are altogether

wanting in the gonopodium of *Quintana* (Text-fig. 7). In both groups the presence of spines on ray 3 (irrespective of their developmental origins) is undoubtedly related to a specialization of segments for sensory function (see Rosen & Gordon, 1953).

When we consider the sum of the morphological evidence it is apparent that the resemblances among *Carlhubbsia, Quintana* and *Girardinus* are mosaic. For example, the suspensorium in *C. kidderi* most closely resembles that of *Girardinus,* less so that of *Quintana,* and least of all that of its congener *C. stuarti.* The gonopodium of *C. kidderi,* on the other hand, most closely approximates that of *C. stuarti,* less so but still significantly that of *Quintana,* and least of all that of *Girardinus.* A consideration of general body form and fin structure suggests additional complex interrelations, for among the recognized species of *Girardinus* there is no well established norm that can be defined precisely. In *Girardinus creolus,* for example, the contours are quite flat whereas in *G. falcatus* and *G. uninotatus* they are sharply angulated. Again, in *G. creolus,* the mouth is distinctly terminal whereas in *G. cubensis* and *G. denticulatus* it is superior; the latter two have previously been separated generically on the basis of further differences in the structure of the mouth parts and position of the gape. In *G. falcatus* the median fins are sharply pointed, whereas in *G. creolus* they are distinctly rounded. In *Quintana* the contours are sharply angulated; in *Carlhubbsia stuarti* the body contours may be so high as to be distinctly rhombic.

But despite the complexity of the many morphological interrelations there is evidence of a central theme. *Carlhubbsia, Quintana* and *Girardinus* have in common the following groups of characters in the gonopodium and gonopodial suspensorium:

(1) a minute recurved terminal hook on ray 3; (2) spines on ray 3; (3) weak retrorse serrae on ray 4p; (4) moderately to well-developed serrae on ray 5p; (5) three highly specialized gonapophyses; (6) uncini almost always present on all three gonapophyses; (7) uncini all lying in same plane; (8) uncini always limited to the proximal portion of the spine's shaft; and (9) distal tips of gonapophyses I and II bent downward to meet the projecting actinosts below.

With recent knowledge of skeletal form and function in the Poeciliidae, primary emphasis is shifted from attention to slight differences in structure to the more significant basic similarities. The array of fundamentally distinctive morphological traits that are shared by *Carlhubbsia, Quintana* and *Girardinus* constitutes strong presumptive evidence of their community of descent.

Zoogeographic Considerations

If *Carlhubbsia, Quintana* and *Girardinus* form a natural group, as is suggested above, then it seems plausible that a common ancestor of these fishes may have evolved from other poeciliid groups in the Atlantic coastal drainages of middle or southern Central America. Invasion of the Greater Antillean islands by a representative stock, by whatever means (possibly some form of waif dispersal), may have occurred as a result of coastal, northward spread on the mainland toward the Yucatan Peninsula. Myers (1938: 359) commented that *Girardinus* is distantly related to Central American types, and must have arisen in Cuba a long time ago. Verification of affinities of *Quintana* and *Girardinus* with the mainland *Carlhubbsia* strengthens the hypothesis that Central America served as the source from which the ancestors of *Quintana* and *Girardinus* made their way to Cuba. We concur with Myers' estimate that the Cuban forms have long been isolated. Although not now clearly demonstrable, the natural group composed of, or including, *Carlhubbsia, Quintana* and *Girardinus* may well have had its genesis in Central America. The geologic and zoogeographic evidence favoring Central America as one of the sources of the Antillean biota has been summarized recently by Darlington (1957).

The West Indian Poeciliidae may be divided into two major groups on the basis of their degree of morphological differentiation from the closest mainland relatives. One of these proves to be a natural grouping, the other artificial.

Group I. *Quintana* and *Girardinus* form a compact natural group. They are distinct generically from all known mainland forms. They occur naturally only on Cuba and the Isle of Pines. *Quintana* is monotypic. *Girardinus* is polytypic, with perhaps 10 species.

Group II. The tribes Poeciliini and Gambusiini recognized in current classification are placed in distinct subfamilies and they are not closely related. Zoogeographically, however, they have many points in common. On the mainland, the Gambusiini occur throughout southern North America and Middle America together with the Poeciliini; the latter in addition have spread across northern South America. In the West Indies the Poeciliini occur naturally in the Greater and Lesser Antilles and on islands off the coast of Nicaragua. Of

the eight genera currently recognized, five certainly occur in, and two, *Limia* and *Curtipenis,* are restricted to, the West Indies. All of the poeciliin genera are closely related, however, and they are only doubtfully distinct. The Gambusiini consist of two or three genera, of which *Gambusia* is widely distributed on the mainland and in the West Indies. In the West Indies *Gambusia* occurs chiefly in the Greater Antilles and in the Bahamas; one species is found on the coastal islands of Nicaragua; another, closely allied to a Cuban form, inhabits the Florida Keys.

In summary, *Quintana* and *Girardinus* are morphologically distinct from all poeciliid genera on the mainland; they are endemic to Cuba and the Isle of Pines. In the Gambusiini and Poeciliini, only two nominal genera are peculiar to the West Indies and these are closely related to widely distributed mainland forms.

The endemism and morphological distinctiveness of *Quintana* and *Girardinus* suggest that they have been derived from one or two early invasions of the West Indies from Central America. In contrast, the less marked differentiation of the West Indian poeciliins and gambusiins and their more general distribution through the Antilles and small coastal islands indicates that they are probably more recent additions to the Antillean fauna. The Gambusiini and Poeciliini probably penetrated the West Indies from Central America, the Poeciliini also from South America, at least into the Windward Islands (Myers, 1938).

Summary

The genus *Carlhubbsia* Whitley contains two known species, *C. kidderi* (Hubbs), from Campeche, Mexico, and El Petén, Guatemala, and *C. stuarti,* n. sp., from the Río Polochic, Guatemala. The genus *Phallichthys* contains three recognized forms, two of which are provisionally ranked as subspecies: *P. amates pittieri* (Meek) from Panama and Costa Rica, *P. a. amates* (Miller) from Honduras and eastern Guatemala, and *P. fairweatheri,* n. sp., from El Petén, Guatemala, and British Honduras. Spot-distribution maps are given for all species: of the two genera only *Carlhubbsia kidderi* and *Phallichthys fairweatheri* are sympatric.

Although there is marked superficial resemblance among the species, it is contended that the two genera represent well separated phyletic lines and owe many common characters, including the asymmetrically folded external genitalia (gonopodia), to parallelism. In morphological assessment, it is noted that certain common features associated with permanent folding of the gonopodium and with the configuration and orientation of the gonapophyses in the gonopodial suspensorium are highly adaptive, and have developed independently in the two lines. More fundamental, presumably antecedent, characters associated with the specialized terminal structures of the gonopodium, the positional relationships and form of the suspensorial uncini and the shape of the gonactinostal complex are interpreted as indicative of the true relationships.

In the species of *Carlhubbsia* and *Phallichthys* dentition is surprisingly uniform. Notable differences among closely related species within other poeciliid groups such as *Poeciliopsis* and *Girardinus,* however, indicate a highly adaptive plasticity and suggest that in this family less weight should be placed on dentitional characters than formerly. In line with the above reasoning *Poecilistes* Hubbs is considered a synonym of *Poeciliopsis* Regan, and in view of notable similarities in basic pattern of gonopodial and suspensorial structures in the group previously called the Girardinini, we propose the reduction of *Glaridichthys* Garman (including *Glaridodon* Garman), *Toxus* Eigenmann, *Dactylophallus* Howell Rivero & Rivas, and *Allodontium* Howell Rivero & Rivas to the synonymy of *Girardinus* Poey. The latter name becomes equivalent to Girardinini, and *Quintana* is similarly of equal scope to Quintanini. There appears, therefore, to be no further necessity for retaining these tribe names, at least with their current limits.

In *Poeciliopsis, Aulophallus* and *Phallichthys,* the basic ornamentaion of the gonopodium is relatively little specialized, although infolding, twisting and consolidation of the structure has proceeded further in *Poeciliopsis* and *Aulophallus* than in *Phallichthys.* Thus the latter genus is set somewhat apart from the others. It is suggested nevertheless that they constitute a natural group which may have arisen from an ancestor in which developmental patterns for gonopodial asymmetry were first becoming established. Since *Phallichthys* contains one species with the gonopodium dextral and one species with it sinistral, and because asymmetry is only moderately developed, it seems likely that the beginnings of this genus, as here defined, predate the time of origin of gonopodial asymmetry in the group as a whole. It is conceded, however, that the specific asymmetric modifications also may well have been independently derived in *Phallichthys,* and *Poeciliopsis* and *Aulophallus.*

In view of the lack of unifying structural bonds among *Phalloptychus, Xenophallus* and *Carlhubbsia,* or between any of these and the

group containing *Phallichthys, Aulophallus* and *Poeciliopsis,* other than that of a folded gonopodium, it is concluded that the Poeciliopsinae, defined on the basis of this character, is an artificial assemblage and should be disrupted.

Carlhubbsia, no longer regarded as closely related to *Phallichthys,* is shown to share many common features of the gonopodium and the suspensorium with the Cuban genera *Quintana* and *Girardinus.* Although these three seem properly separated at the generic level they form a natural group in the family. Thus, *Carlhubbsia* provides mainland representation of the Antillean group, and points to the probability that the ancestors of the Cuban genera came from Middle America. Such ancestors probably had symmetrical gonopodia, since those of *Girardinus* are symmetrical; in *Quintana* only a few segments near the tip are slightly twisted sinistrally. These genera are notably more sharply differentiated from *Carlhubbsia* than are other Antillean poeciliids from their mainland relatives. This suggests the likelihood that *Quintana* and *Girardinus* stem from an earlier invasion of the islands than do the poeciliins and gambusiins that live there.

Acknowledgments

This attempt to advance our knowledge of *Carlhubbsia* and *Phallichthys* would have been fruitless except for the wealth of new and unreported collections of four of the five recognized forms. We deeply appreciate the privilege of studying these materials and for their contribution thank the collectors: the Rev. Gerald Fairweather, Drs. Myron Gordon, Carl L. Hubbs, Henry van der Schalie and Laurence C. Stuart. Most of the previously known specimens of these genera have been available on loan. We acknowledge the cooperation of Loren P. Woods, Chicago Natural History Museum, and of Drs. Leonard P. Schultz and Ernest A. Lachner, United States National Museum.

For their many suggestions during the work and for invaluable discussion and criticism of the manuscript we sincerely thank Mr. James W. Atz, and Drs. Charles M. Breder, H. Clark Dalton, Myron Gordon, Robert R. Miller, Bobb Schaeffer and Laurence C. Stuart. One of us (Rosen) wishes especially to thank Dr. Myron Gordon for the opportunity to carry on the bulk of the investigation in the Genetics Laboratory of the New York Zoological Society and for his time and interest generously given in discussions of the many problems involved.

The maps and Text-fig. 6 were prepared by Mrs. Elizabeth Anthony, and the photographs were taken by William L. Brudon, both on the staff of the University of Michigan Museum of Zoology. Some of the X-rays and the prints made from them were prepared by Elwood Logan of the American Museum of Natural History. We thank all of them.

Addendum

While the present manuscript was in press, there appeared in the Proceedings of the American Philosophical Society (102 [3]: 281-320, 1958) an article by L. R. Rivas entitled "The origin, evolution, dispersal, and geographical distribution of the Cuban poeciliid fishes of the Tribe Girardinini." Based on our independent morphological studies, we find that our views are at variance with those of Rivas in questions of the origin, relationships and taxonomy of the Cuban endemic poeciliids. We maintain without emendation our original interpretations. For further discussion of the zoogeographic problems the reader is referred to the recent reviews of W. P. Woodring (Bull. Geol. Soc. Amer., 65: 719-732, 1954), "Caribbean land and sea through the ages," and Darlington (1957).

Literature Cited

Alfaro, Anastasio

1935. Investigaciones cientificas. San José, Costa Rica, pp. 1-317.

Behre, Ellinor H.

1928. A list of the fresh water fishes of western Panama between long. 81° 45′ and 83° 15′ W. Ann. Carnegie Mus., 18 (2): 305-328, pls. 18-19.

Breder, Charles M., Jr.

1925. Notes on fishes from three Panama localities: Gatun Spillway, Rio Tapia and Caledonia bay. Zoologica, 4 (4): 137-158, figs. 33-38.

Darlington, Philip J.

1957. Zoogeography: the geographical distribution of animals. John Wiley & Sons, Inc., New York, 675 pp.

Gosline, William A.

1949. The sensory canals of the head in some cyprinodont fishes, with particular reference to the genus *Fundulus.* Occ. Papers Mus. Zool. Univ. Mich., 519: 1-17, pls. 1-2, fig. 1.

Henn, Arthur W.

1916. On various South American poeciliid fishes. Ann. Carnegie Mus., 10 (1-2): 93-142, pls. 18-21, figs. 1-17.

HILDEBRAND, SAMUEL F.

1930. Notes on a collection of fishes from Costa Rica. Copeia (1): 1-9.

1938. A new catalogue of the fresh-water fishes of Panama. Field Mus. Nat. Hist., Publ. 425, Zool. Ser., 22 (4): 217-359, figs. 2-13.

HOWELL RIVERO, LUIS

1946. El esqueleto sexual de los peces de la familia Poeciliidae. Mem. Soc. Cubana Hist. Nat., 18 (2): 133-152, figs. 1-16.

HOWELL RIVERO, LUIS, & LUIS RENÉ RIVAS

1944. Studies of cyprinodont fishes. Two new genera of the tribe Girardinini, from Cuba. Torreia (12): 1-19, pls. 1-2, figs. 1-9.

HUBBS, CARL L.

1924. Studies of the fishes of the order Cyprinodontes. Misc. Publ. Mus. Zool. Univ. Mich., 13: 1-31, pls. 1-4.

1926. Studies of the fishes of the order Cyprinodontes. VI. Material for a revision of the American genera and species. Misc. Publ. Mus. Zool. Univ. Mich., 16: 1-86, pls. 1-4.

1934. Studies of the fishes of the order Cyprinodontes. XIII. *Quintana atrizona,* a new poeciliid. Occ. Papers Mus. Zool. Univ. Mich., 301: 1-8, pl. 1.

1936. Fishes of the Yucatan Peninsula. Carnegie Inst. Wash., Publ. 457: 157-287, pls. 1-15, fig. 1.

1950. Studies of cyprinodont fishes. XX. A new subfamily from Guatemala, with ctenoid scales and a unilateral pectoral clasper. Misc. Publ. Mus. Zool. Univ. Mich., 78: 1-28, pls. 1-4, map 1.

HUBBS, CARL L., & LAURA C. HUBBS

1945. Bilateral asymmetry and bilateral variation in fishes. Papers Mich. Acad. Sci., Arts, and Letters, 30 (1944): 229-310, pl. 1, figs. 1-2.

HUBBS, CARL L., & ROBERT RUSH MILLER

1954. Studies of cyprinodont fishes. XXI. *Glaridodon latidens,* from northwestern Mexico, redescribed and referred to *Poeciliopsis.* Zoologica, 39 (1): 1-12, pl. 1, text-fig. 1.

HUBBS, CARL L., & C. L. TURNER

1939. Studies of the fishes of the order Cyprinodontes. XVI. A revision of the Goodeidae. Misc. Publ. Mus. Zool. Univ. Mich., 42: 1-80, pls. 1-5.

JORDAN, DAVID STARR, BARTON WARREN EVERMANN & HOWARD WALTON CLARK

1930. Check list of the fishes and fishlike vertebrates of North and Middle America north of the northern boundary of Venezuela and Colombia. Rept. U. S. Comm. Fish., 1928 (2): 1-670.

MEEK, SETH EUGENE

1912. New species of fishes from Costa Rica. Field Mus. Nat. Hist., Publ. 163, Zool. Ser., 10 (7): 69-75.

1914. An annotated list of fishes known to occur in the fresh waters of Costa Rica. Field Mus. Nat. Hist., Publ. 174, Zool. Ser., 10 (10): 101-134.

MEEK, SETH E., & SAMUEL F. HILDEBRAND

1916. The fishes of the fresh waters of Panama. Field Mus. Nat. Hist., Publ. 191, Zool. Ser., 10 (15): 217-374, pls. 6-32, figs. 1-10.

MILLER, NEWTON

1907. The fishes of the Motagua River, Guatemala. Bull. Amer. Mus. Nat. Hist., 23: 95-123, figs. 1-6.

MILLER, ROBERT RUSH

1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Misc. Publ. Mus. Zool. Univ. Mich., 68: 1-155, pls. 1-15, figs. 1-5, maps 1-3.

1955. A systematic review of the Middle American fishes of the genus *Profundulus.* Misc. Publ. Mus. Zool. Univ. Mich., 92: 1-64, pls. 1-9, figs. 1-6, maps 1-3.

1956. A new genus and species of cyprinodontid fish from San Luis Potosí, Mexico, with remarks on the subfamily Cyprinodontinae. Occ. Papers Mus. Zool. Univ. Mich., 581: 1-17, pls. 1-2, figs. 1-2.

1957. Utilization of X-rays as a tool in systematic zoology. Syst. Zool., 6 (1): 29-40, figs. 1-4.

MYERS, GEORGE S.

1925. Results of some recent studies on the American killifishes. Fish Culturist, 4: 370-371.

1935. An annotated list of the cyprinodont fishes of Hispaniola, with descriptions of two new species. Zoologica, 10 (3): 301-316, figs. 273-279.

1938. Fresh-water fishes and West Indian zoogeography. Ann. Rept. Smithsonian Inst., 1937: 339-364, pls. 1-3.

REGAN, C. TATE

1913. A revision of the cyprinodont fishes of the subfamily Poeciliinae. Proc. Zool. Soc. London, 2: 977-1018, pls. 99-101, text-figs. 168-173.

ROSEN, DONN ERIC, & MYRON GORDON

1953. Functional anatomy and evolution of male genitalia in poeciliid fishes. Zoologica, 38 (1): 1-47, pls. 1-4, text-figs. 1-47.

SCRIMSHAW, NEVIN S.

1944. Superfetation in poeciliid fishes. Copeia (3): 180-183.

1945. Embryonic development in poeciliid fishes. Biol. Bull., 88 (3): 233-246.

1946. Egg size in poeciliid fishes. Copeia (1): 20-23.

Turner, C. L.

1940. Superfetation in viviparous cyprinodont fishes. Copeia (2): 88-91.

Whitley, G. P.

1951. New fish names and records. Proc. Royal Zool. Soc. New South Wales, 1949-50 (1951): 61-68, figs. 8-10.

Woods, Loren P., & Robert Inger

1957. The cave, spring, and swamp fishes of the family Amblyopsidae of the central and eastern United States. Amer. Mid. Nat., 58 (1): 232-256.

EXPLANATION OF THE PLATES

Plate I

Carlhubbsia stuarti, n. sp., Río Polochic, near Panzos, Guatemala.

Fig. 1. Holotype, UMMZ 146084, an adult male 38.3 mm. in standard length.

Fig. 2. Allotype, UMMZ 172455, an adult female 50.5 mm. long. Photographs by William L. Brudon.

Plate II

Carlhubbsia kidderi (Hubbs), Arroyo Subín, tributary to Río de la Pasión, El Petén, Guatemala (UMMZ 144217).

Fig. 3. Adult male, 21.0 mm. in standard length.

Fig. 4. Adult female, 36.5 mm. long. Retouched photographs by William L. Brudon.

Plate III

Phallichthys amates pittieri (Meek), Siquirres, Limón, Costa Rica (USNM 92158).

Fig. 5. Adult male, 23.5 mm. in standard length.

Fig. 6. Adult female, 30.5 mm. long. Photographs by William L. Brudon.

Plate IV

Phallichthys amates amates (Miller), tributary to Río San Alejo, San Alejo, Atlantida, Honduras (UMMZ 173221).

Fig. 7. Adult male, 25.5 mm. in standard length.

Fig. 8. Adult female, 32.0 mm. long. Photographs by William L. Brudon.

Plate V

Phallichthys fairweatheri, n. sp., Río San Pedro de Mártir, El Petén, Guatemala.

Fig. 9. Holotype, UMMZ 172456, an adult male 29.7 mm. in standard length.

Fig. 10. Allotype, UMMZ 172457, an adult female, 33.3 mm. long. Photographs by William L. Brudon.

Plate VI

Fig. 11, A-F. Radiographs of the gonopodial suspensoria of six adult males of *Phallichthys fairweatheri,* n. sp. (UMMZ 144186), showing variability in form, orientation, and development of various structures. The gonactinostal complex is typically slender in **A** and **B**, somewhat less so in **E** and **F**, and unusually broad in **C** and **D**. The dorsal tip of the ligastyle lies beneath the 10th vertebra in A, D and E, and between the 10th and 11th vertebrae in B, C and F. Uncini near the base of gonapophysis I are typically well developed in A-D, small in E, and absent in F. Uncini near the tip of gonapophysis III are poorly developed or obsolescent in A and D-F, well developed in B and C.

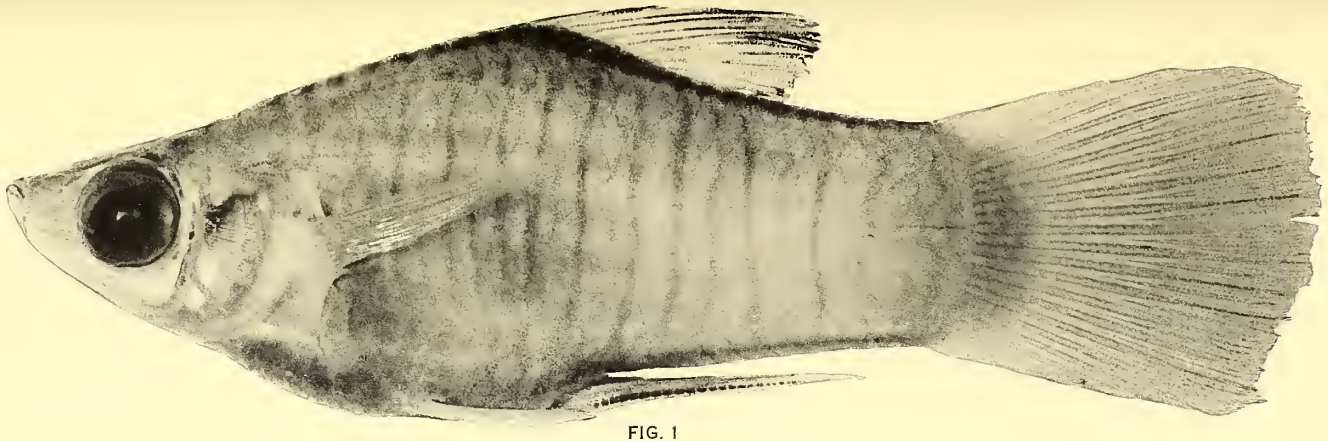


FIG. 1

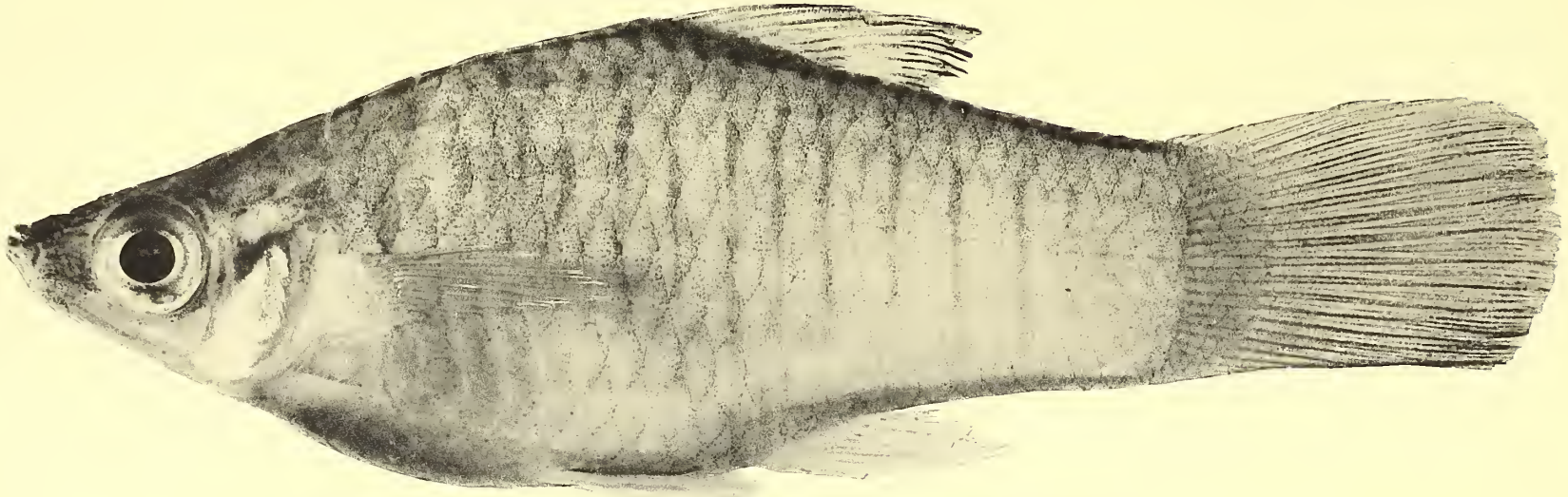


FIG. 2

MIDDLE-AMERICAN POECILIID FISHES OF THE GENERA CARLHUBBSIA AND PHALLICHTHYS, WITH DESCRIPTIONS OF TWO NEW SPECIES

FIG. 3

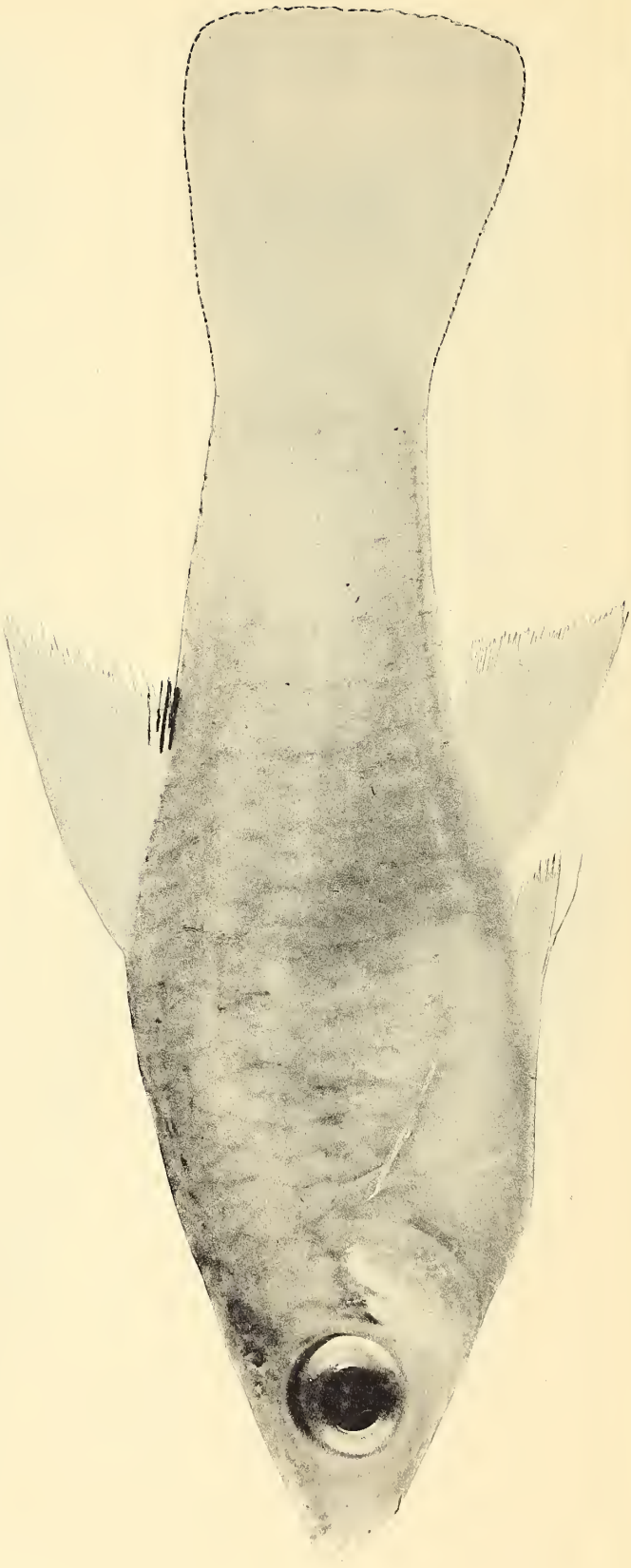


FIG. 4

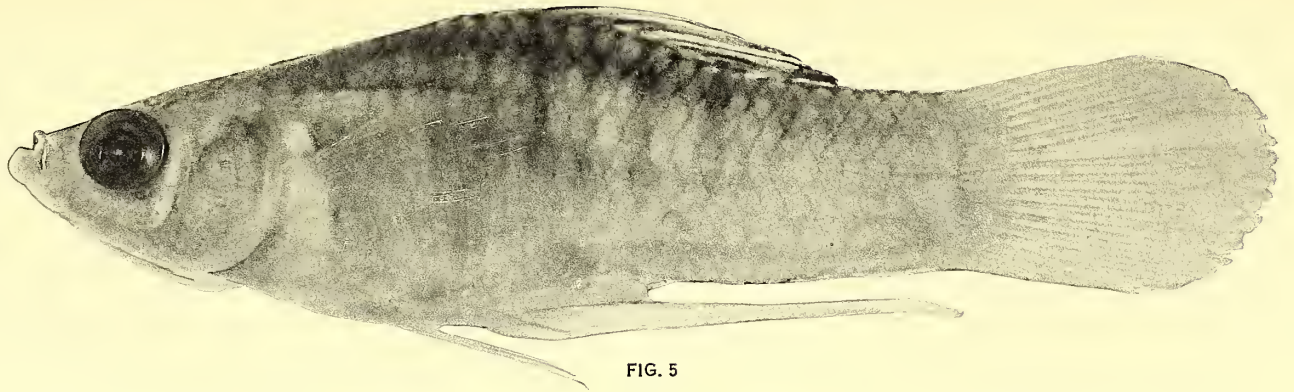


FIG. 5

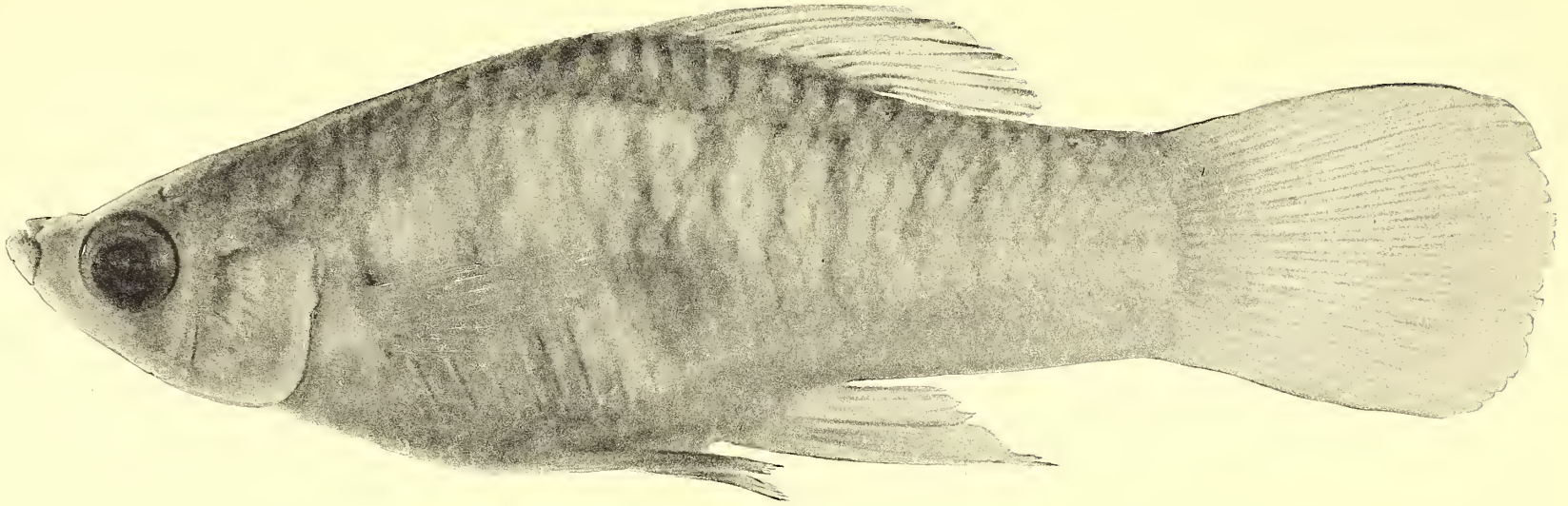


FIG. 6

MIDDLE-AMERICAN POECILIID FISHES OF THE GENERA CARLHUBBSIA AND PHALLICHTHYS, WITH DESCRIPTIONS OF TWO NEW SPECIES

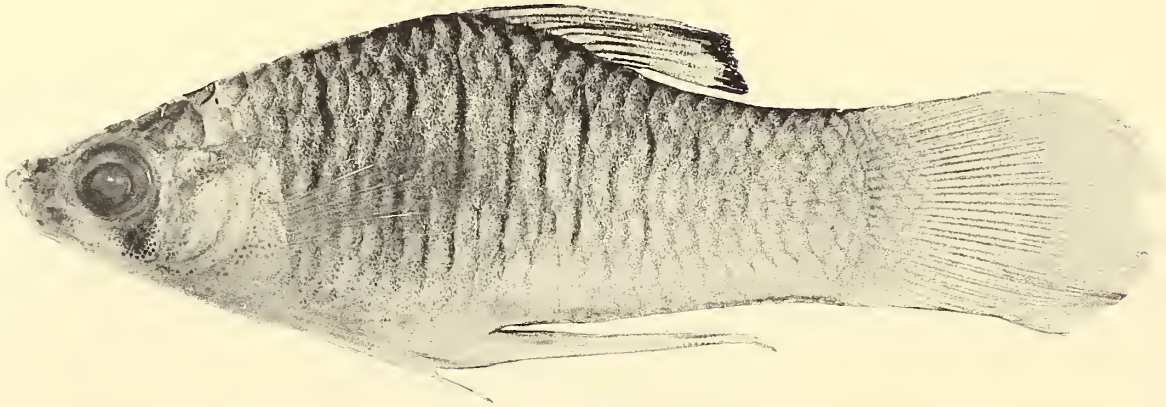


FIG. 7

FIG. 8

MIDDLE-AMERICAN POECILIID FISHES OF THE GENERA CARLHUBBSIA AND PHALLICHTHYS, WITH DESCRIPTIONS OF TWO NEW SPECIES

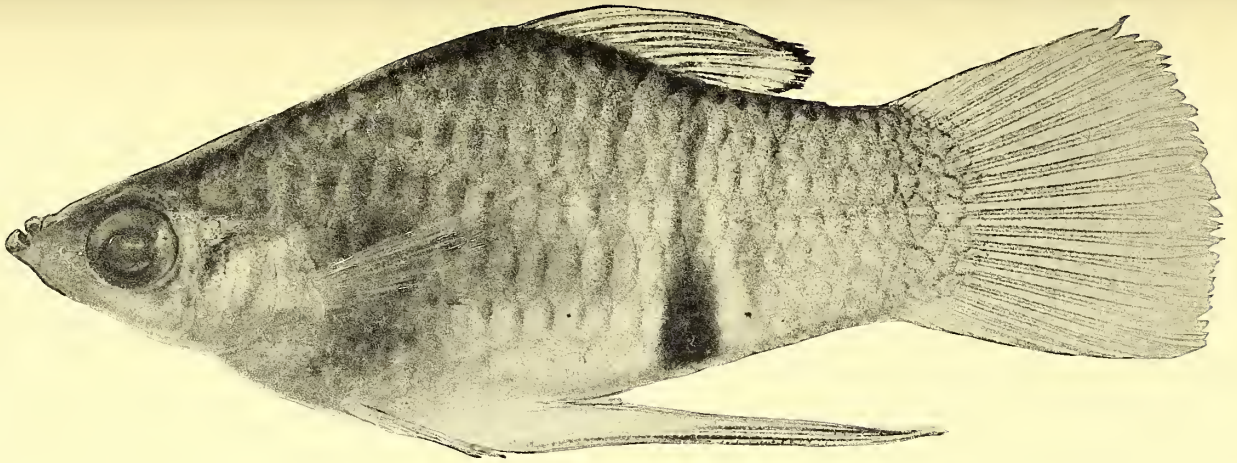
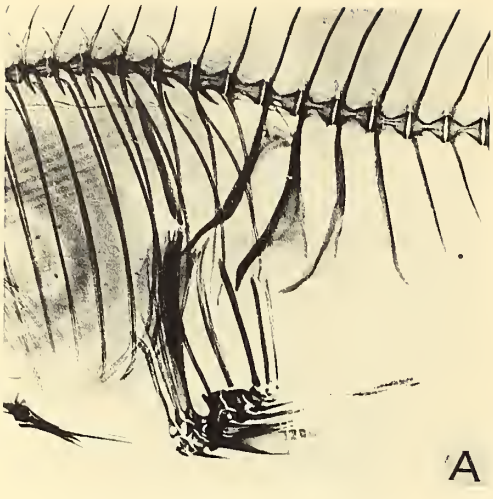
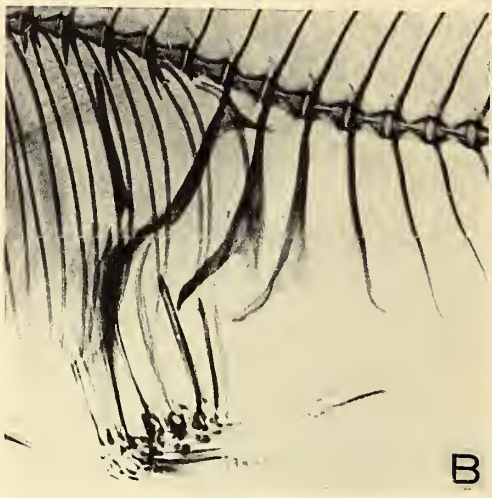


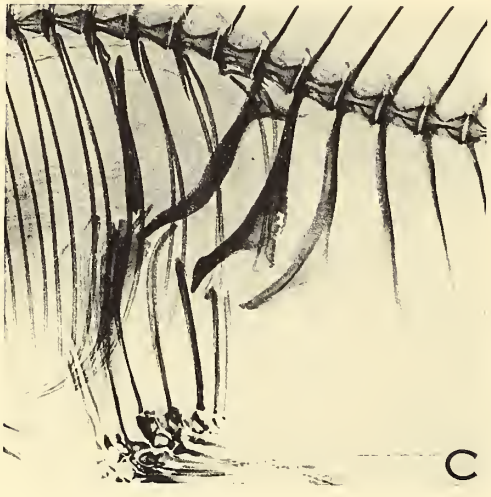
FIG. 9

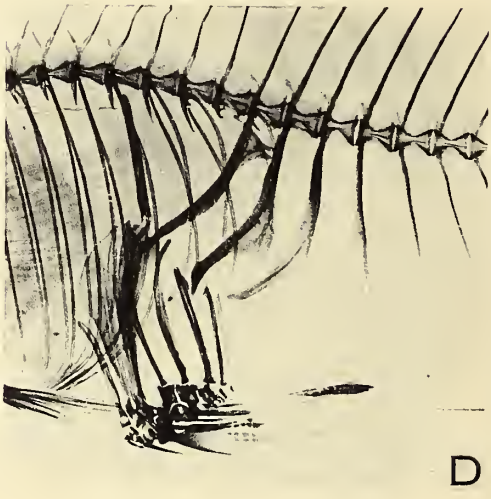
FIG. 10

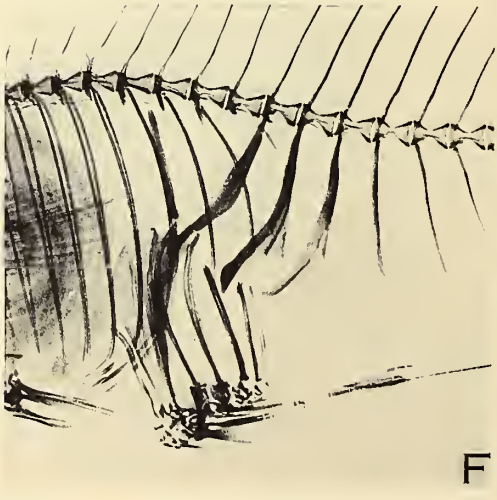
MIDDLE-AMERICAN POECILIID FISHES OF THE GENERA CARLHUBBSIA AND PHALLICHTHYS, WITH DESCRIPTIONS OF TWO NEW SPECIES











MIDDLE-AMERICAN POECILIID FISHES OF THE GENERA CARLHUBBSIA AND PHALLICHTHYS, WITH DESCRIPTIONS OF TWO NEW SPECIES

NEW YORK ZOOLOGICAL SOCIETY

GENERAL OFFICE
30 East Fortieth Street, New York 16, N. Y.

PUBLICATION OFFICE
The Zoological Park, New York 60, N. Y.

OFFICERS

PRESIDENT	VICE-PRESIDENTS	SECRETARY	TREASURER
Fairfield Osborn	Alfred Ely Laurance S. Rockefeller	Harold J. O'Connell	David H. McAlpin

SCIENTIFIC STAFF:

John Tee-Van ..... *General Director*

James A. Oliver... *Director, Zoological Park*

Christopher W. Coates.. *Director, Aquarium*

ZOOLOGICAL PARK

Grace Davall..... *Assistant Curator, Mammals and Birds*

Joseph A. Davis, Jr... *Assistant Curator, Mammals*

William G. Conway.. *Curator, Birds*

James A. Oliver.... *Curator, Reptiles*

Charles P. Gandal .. *Veterinarian*

Lee S. Crandall..... *General Curator Emeritus*

William Beebe..... *Honorary Curator, Birds*

AQUARIUM

James W. Atz..... *Associate Curator*

Carleton Ray..... *Assistant to the Director*

Ross F. Nigrelli..... *Pathologist & Chairman of Department of Marine Biochemistry & Ecology*

C. M. Breder, Jr..... *Research Associate in Ichthyology*

Harry A. Charipper... *Research Associate in Histology*

Homer W. Smith..... *Research Associate in Physiology*

GENERAL

William Bridges .. *Editor & Curator, Publications*

Sam Dunton ..... *Photographer*

Henry M. Lester.. *Photographic Consultant*

DEPARTMENT OF TROPICAL RESEARCH

William Beebe..... *Director Emeritus*

Jocelyn Crane..... *Assistant Director*

David W. Snow ..... *Resident Naturalist*

Henry Fleming ..... *Entomologist*

John Tee-Van ..... *Associate*

William K. Gregory.... *Associate*

AFFILIATES

L. Floyd Clarke ..... *Director, Jackson Hole Biological Research Station*

SCIENTIFIC ADVISORY COUNCIL

A. Raymond Dochez	Caryl P. Haskins
Alfred E. Emerson	John S. Nicholas
W. A. Hagan	

EDITORIAL COMMITTEE

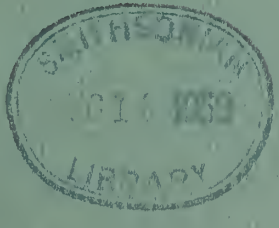
Fairfield Osborn, *Chairman*

James W. Atz	William G. Conway
William Beebe	Lee S. Crandall
William Bridges	James A. Oliver
Christopher W. Coates	John Tee-Van

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 44 • PART 2 • JULY 20, 1959 • NUMBERS 2 TO 5



PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

2

Studies on the Histology and Histopathology of the Rainbow Trout, *Salmo gairdneri irideus*. II. Effects of Induced Inflammation and Cortisone Treatment on the Digestive Organs[1]

Eva Lurie Weinreb

Department of Zoology, University of Wisconsin, Madison

(Plates I & II)

THIS study was undertaken to determine whether physiological responses to induced inflammation in a poikilothermic vertebrate such as the trout are similar to those found in common laboratory animals. Responses of the digestive tract tissues, particularly those of the stomach and intestines, were studied following chemically and physically induced inflammation. Effects of cortisone on inflammation and wound healing were also determined. The histology of the digestive organs under such experimental conditions was compared with the normal histology previously described (Weinreb & Bilstad, 1955).

Irritants used in different mammals as a means of producing sterile inflammation have included turpentine (Menkin, 1940.1; Cartwright *et al.*, 1951; Shapiro *et al.*, 1951; Moon & Tershakovec, 1951; Spain *et al.*, 1952), croton oil (Clark & Clark, 1920; Michael & Whorton, 1951) and hot water (Menkin, 1933). The relation between the leukocytes, reticulo-endothelial system, and adrenal cortex following turpentine administration was discussed by Cartwright and co-workers (1951).

The influence of various hormones on inflammation has been reported. The effects of adrenal cortical extract were compared with those of other steroid hormones (Menkin, 1942, 1951.1, 1951.2), and the inhibitory effect of cortisone described (Michael & Whorton, 1951; Shapiro *et al.*, 1951; Spain *et al.*, 1952; Rebuck & Mellinger, 1953). Responses to large doses of both cortisone and ACTH were also determined (Robinson & Smith, 1953). The inhibitory effect of steroid hormones and ACTH on granulation tissue and wound healing has been reported by Taubenhaus (1953), Taubenhaus and co-workers (1949, 1952), Baker & Ingle (1948), Castor & Baker (1950) and Baker & Whitaker (1950).

Almost all of the work cited above refers to homoiothermic animals, and few detailed studies have been reported for poikilothermic vertebrates.

The author is indebted to Dr. Nellie M. Bilstad for her guidance, to Dr. Stanley Weinreb for preparation of the photomicrographs and critical reading of the manuscript, and to Mr. Henry Eichhorn, Jr., for technical assistance.

This work has been supported by funds made available by the Wisconsin Alumni Research Foundation.

Materials and Methods

Inflammation was initiated chemically by means of intraperitoneal injections of turpentine, and physically by surgical trauma. Four to six rainbow trout were used for each experiment. The animals were injected as described previously (Weinreb, 1958), and tissue was removed immediately following collection of blood samples, at the time intervals indicated. Samples of cardiac stomach and ascending intestine, at the level of the pyloric caeca and pancreas, were excised. Tissues were fixed in Helly's solution and stained with hematoxylin-eosin, hematoxylin - eosin - azure and periodic acid - Schiff

[1]A revised portion of the thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Wisconsin.

(PAS) reagent. Two controls were used with PAS, namely salivary digestion and omission of the oxidizing agent. The hematoxylin-eosin-azure technic was that outlined by Yokoyama (1947). The latter procedure was substituted for H & E as the technic of choice, because of its greater differentiation of blood elements in inflammation.

Turpentine-injected trout were also given cortisone, as described previously (Weinreb, 1958). An average of four trout were used per experiment, and sections of stomach and intestine were excised. In addition, one animal received 0.4 cc. Thorotrast (Heyden Chemical Corp., New York) followed by turpentine injection at 24 hours and sacrificed at 30 hours. Phagocytic activity coincident with inflammation was thus compared with similar activity in the normal animal.

Surgical trauma was induced by an incision in the descending intestine. The animal was anaesthesized in dilute urethane and a small incision made in the ventral body wall just anterior to the pelvic fin, at the level of the spleen, after taking precautions to prevent drying. After lifting the intestinal limb through the previous incision, a small cut was made in the intestinal wall. The intestine was carefully replaced and the adominal incision closed by a skin clamp. The entire operation lasted but a few minutes and the trout resumed normal swimming upon return to the tank. The trout were sacrificed and the region of the intestinal incision excised after 12, 24, 48 and 72 hours. The 12- and 24-hour groups each consisted of 6 trout, half of which had been given injections of 1 mg./cc. cortisone 24 hours earlier. Two trout were maintained 48 hours and one for 72 hours. Tissues were fixed in Helly's solution and stained with hematoxylin-eosin-azure and PAS.

Results

Intraperitoneally - injected turpentine caused distress to some of the trout, resulting in difficult respiration and sluggishness. Animals exhibiting increased weakness within one to two hours constituted part of the early mortality group. These animals all had pale gills, attributable to shunting of blood to the sites of injection and inflammation, as well as shock-induced constriction of branchial vessels.

The strong odor of turpentine was prominent in the body fluid and tissues at autopsy. More than usual amounts of blood-tinged fluid were found in the body cavity, accompanied by a whitening and loss of firmness of the fat bodies around the stomach and intestine. Although the viscera appeared paler, there was dilation of the blood vessels of the visceral peritoneum and inflammation of the parietal peritoneum. The lumen of the stomach contained a white fluid. A thicker yellow-orange fluid was found in the intestine; its color probably was the result of increased bile content.

Trout injected with cortisone and turpentine concurrently, exhibited similar distress. Animals which had been injected with cortisone 24 hours previously showed less effect from the turpentine. In addition, the peritoneum and viscera of trout pretreated with cortisone showed almost no change at autopsy, while the former group exhibited gross changes to a lesser degree similar to those in animals given turpentine alone.

Marked histological changes were observed. Lesions were most pronounced in the cardiac stomach and were also evident in the intestine, caeca and pancreas. Microscopic lesions in the stomach were noted as early as one hour following turpentine injection. The submucosa exhibited edema, hyperemia and fibroblastic proliferation. The surface epithelium was vacuolated and granular, while the serous cells of the cardiac glands showed an increase in zymogen granules and secretory activity. Edema, dilation and congestion of blood vessels, with endothelial swelling, continued throughout the first 6 hours. After 3 hours an increase in the number of cells and the amount of intracellular granulation was observed in the stratum granulosum. The lumen was lined by an exudate containing blood cells, bacteria and debris. A purulent exudate covered the serosa. The reaction was similar after 5 hours, with additional leukocytic and macrophagic infiltration of the submucosa.

The increase in fibroblasts and granule cells, the latter layer being more than double (5-6 cells deep) the normal thickness, was most marked after 7 hours (Pl. I, Fig. 1). This was accompanied by an increase in collagen and thickening of the mucosal basement membranes. After 7 hours the edema of the submucosa and muscle coats lessened. Endothelial swelling continued taking on a syncitial appearance. The surface epithelium showed some necrosis and sloughing at the tips of the rugae. After 10 hours leukocytic infiltration of the tunica propria and submucosa were noted in addition to the above observations.

During the first 10 hours the heterophil was the prominent leukocyte, with relatively few lymphocytes present. It was noted that PAS staining sharply differentiated between heterophils and lymphocytes. Heterophil cytoplasm stained red-purple (with loss in intensity following sali-

vary digestion) while lymphocytes remained unstained.

Edema of the submucosa and muscle coats was still extensive after 24 hours. Blood and lymph vessel dilation continued, with margination of leukocytes. Fibroblasts remained abundant, but the granule cell layer regressed to only two cells deep.

Very prominent lesions, predominantly in the submucosa (Pl. I, Fig. 2), were noted after 24 hours. The largest of these lesions were tubercle-like structures similar to those associated with infectious granulomas. They consisted of a necrotic center surrounded by large immature cells, resembling phagocytic epithelioid cells, enclosed by fibroblasts and collagenic strands (Pl. I, Fig. 3). The presence of granule cells among the immature cells was not uncommon. Various stages in "tubercle" formation and degeneration were found, the later stage resembling the Langhans type of foreign body giant cell. Another prominent lesion derived from small blood vessels resulted from a fusion of swollen and degenerating endothelial cells. The lumina of these vessels were occluded by laminated concretions.

Over the 24-hour period the surface epithelium continued to be granular and vacuolated, while the serous cells returned to normal. By 48 hours the edema and congestion in the blood vessels had lessened and the tubercle-like lesions were less frequent. Areas of necrosis, predominantly infiltrated by lymphocytes, were prominent. Inflammation had greatly subsided after 60 hours. Return to normal was apparent in the stomach by 72 hours.

Lesions in the ascending intestine, although more marked than in the caeca, were less prominent than in the stomach. Intestinal and caecal epithelium exhibited some necrosis, accompanied by serosal edema, as early as one hour following turpentine injection. Increased liquefaction and leukocytic infiltration were evident after 3 hours and continued for the initial 6 hours. Necrosis was attributed to enzymatic digestion, particularly by pancreatic enzymes. Goblet cells in the intestines and caeca were greatly dilated after 7 hours and remained so for 48 hours. Granule cells and inflammatory cells were prominent after 7 hours, the number of granule cells remaining high over the entire 72-hour period.

Edema of the mucosal connective tissue and sloughing of the apical epithelium were extensive around 10 hours, with the swelling lasting the 3-day period. Heterophil infiltration of the necrotic areas was marked prior to 48 hours, followed by lymphocytes after the second day. Peritonitis was also evident. The stratum compactum of the digestive tract showed little response to the irritant during this time. By 72 hours more normal tissue architecture was evident, although edema and inflammation of the tunica propria persisted.

Turpentine caused destruction of fat and overlying pancreatic acini during the first 3 hours. Liquefaction and leukocytic invasion were marked by 10 hours. Thickening of the basement membranes in the larger Islets was the sole lesion noted in these areas. Although necrotic foci persisted, normal tubular structure was present in the pancreas at 60 hours.

Cortisone administered to trout previously injected with turpentine markedly altered the histological picture. Tissues excised 24 hours after concurrent injections of cortisone and turpentine did not exhibit the degree of lesions resulting from turpentine alone. The gastric lesions in these trout resembled those noted 7 hours after turpentine alone (Pl. I, Fig. 4). Granule cell number in these trout increased in the tunica propria and submucosa. Although submucosal edema occurred, minimal inflammatory response was found with concurrent injections. Tissues from trout given cortisone 24 hours prior to turpentine exhibited no apparent inflammatory response, although in control animals, which received cortisone alone, slight increases in granule cells were noted after 6 and 24 hours.

In the trout injected with Thorotrast 24 hours prior to turpentine, the distribution of Thorotrast after 30 hours was comparable to that found after 3 days in normal animals (Weinreb & Bilstad, 1955). In addition, a greater concentration of foreign matter was present in the blood and lymph vessels at this earlier time. Both the rate of pickup and amount of Thorotrast concentrated in the macrophages indicated increased phagocytosis coincident with inflammation. It was also noted that the loci of Thorotrast particles were rendered more visible after PAS staining.

Trauma produced by cutting the descending intestine did not appear to produce any noticeable effects on the trout behavior, and no mortalities resulted. On autopsy, however, gross differences were visible between cortisone-treated and untreated trout. In cortisone-treated trout the wound remained open, whereas in untreated animals the incision was difficult to find. In the latter group the cut area was hidden by thick exudate. Binding fibrinous exudate was lacking, or present to a lesser degree, after cortisone administration. Increased body fluid and signs of

inflammation were also more evident in the untreated group.

Tissue excised from the untreated trout 12 hours after operation exhibited acute inflammation, particularly in the tunica propria, and hyaline degeneration of the muscle coat near the cut. Tissue from the cortisone-treated group, excised after the same time interval, showed moderate inflammation with minimal hyperemia and granulation tissue formation. In addition, there was notable mucosal necrosis. Tissue removed after 24 hours showed comparable contrast. Intestine from untreated animals exhibited prominent granulation tissue in the mucosal folds (Pl. II, Fig. 5), whereas following cortisone no indication of healing was seen (Pl. II, Fig. 6). After 48 hours, granulation tissue was more extensive, some peritonitis was noted and there was an increase in granule cell number. In the one animal seen after 72 hours, tissue destruction and acute inflammation were extensive (Pl. II, Fig. 7); secondary infection was superimposed upon the original inflammation.

Discussion

Early signs of inflammation in the digestive organs were in direct response to absorption of the irritant from the body cavity. The speed of reaction was attributed to the rapidity of turpentine penetration and the physiological response of the trout. The characteristic signs of inflammation are directly associated with the presence of an irritant in the tissues; the turpentine, *per se,* does not directly initiate the reaction. Similar responses, using other irritants, were noted by Moon & Tershakovec (1951), who attributed this response to tissue chemotaxis.

Menkin (1940.2) previously had proposed that different factors released in the exudate stimulated leukocytosis (leukocytosis-promoting factor, LPF), followed by cell migration with increased capillary permeability (leukotaxine). He also reported a pH shift of the exudate from alkaline during the acute stage to acid in later stages. This pH shift is probably associated with the particular leukocytes present at that stage, namely polymorphonuclear or mononuclear leukocytes. Applying Menkin's concepts to the trout, the early responses of the tissues would be initiated by a chemotactic exudate followed by evidence of inflammation. This sequence was inhibited by cortisone.

Inhibition of inflammation by various steroid hormones, and ACTH, was reported by Menkin (1940.2, 1942, 1951.1, 1951.2), who later (1954) proposed that the anti-inflammatory mechanism acted on the cellular level at the site of inflammation. Thomas (1953) had earlier suggested that cortisone impaired the functioning of the reticulo-endothelial system.

The reaction of the trout stomach, seen 24 hours after concurrent injections of turpentine and cortisone, was unlike the response noted 24 hours after turpentine alone, resembling instead the responses seen 7 hours after turpentine. Similar delays in inflammatory response due to cortisone were reported by Michael & Whorton (1951) and Spain and co-workers (1952). Trout given cortisone 24 hours prior to turpentine exhibited complete inhibition of inflammation. It appears that the presence of the hormone before the onset of inflammation may successfully inhibit formation of chemotactic agents, and/or inhibit the ability of the reticulo-endothelial system to respond.

The response of the blood elements of the trout seen in the tissues during inflammation corresponds with the changes noted in the circulating leukocytes (Weinreb, 1958). The heterophilia characteristic of the early stages of inflammation is correlated with the prevalence of these cells in the tissues during the acute stage, and attributed to increased output of immature cells under the influence of LPF. Increase in lymphocytes in the tissues is correlated with their decrease in the circulation following augmented migration without corresponding lymphocyte production. In trout given cortisone such leukocytic infiltration was inhibited and, in addition, few macrophages were present in the tissues. Dougherty & Schneebeli (1950) reported less neutrophilic and macrophagic response in inflammation in cortisone-treated mice, while Rebuck & Mellinger (1953) reported similar effects following cortisone treatment in man; the latter group also suggested injury to the organ sources of the leukocytes and macrophages.

Cellular changes in the trout organs were notable in the macrophages, granule cells and fibroblasts. Increased macrophage activity was demonstrated by rapid uptake of Thorotrast. Gordon & Katsh (1949) reported a similar increase in phagocytosis of Thorotrast in rats subjected to starvation, such activity being reduced in animals with adrenal insufficiency and enhanced by administration of cortical hormone. A correlation between increased activity of the R-E elements with stress and adrenal cortical stimulation was suggested. The response of the rainbow trout to induced inflammation, and that of rats under inanition, is comparable and attribut-

able to adrenal cortical stimulation of the R-E elements.

The changing numbers of granule cells in the stomach and intestine during inflammation indicates a cyclic activity. Three hours after turpentine injection the number of cells in the cardiac stomach increases above normal and remains so for 24 hours, reaching a peak at 7 hours. This increase is not due to migration from other parts of the digestive tract, equivalent numbers being present throughout its length. The number of cells is also slightly higher after cortisone injection. This increase appears to be in direct response to the irritant, probably mediated by way of the adrenal cortex.

The drop in granule cell number at 24 hours is coincident with the appearance of the submucosal tubercle-like lesions. Although granule cells are noted among the immature cells and fibroblasts, they are not directly involved in tubercle formation. The exact role of these cells is still undetermined. As inflammation subsided at about 60 hours, the number of cells returned to normal or slightly above normal. In trout given cortisone concurrently with turpentine, this cyclic response is absent. In these fish, after 24 hours, the cell number is still above normal and no tubercles are present. It appears that cortisone delays the reaction, the 24-hour response being like that seen at 7 hours, or blocks the mechanism eliciting the granule cell decrease.

The tubercle-like lesions are due to cellular destruction following introduction of the irritant. The immature epithelioid-like cells, though of indefinite origin, may be associated with leukocyte response by way of a common stem cell present in the tissues and hemopoietic organs. Cell fusion, forming giant cells, is probably a stage in degeneration involving changes in the cell membrane and cytoplasm. The important factor in inflammation, in all animals, is probably not a specific lesion or particular site, but the response of connective tissue elements, in general, to the presence of an irritant, with resultant phagocytic activity.

The necrosis noted in the intestine, caeca and pancreas in the early stages of inflammation is attributed to enzymatic digestion of injured cells. The minor destruction seen at the base of the intestinal folds, compared to that at the apices, is understood in view of the greatly increased goblet cell activity in these areas. The minimal effect in the caeca, compared to that in the intestine, is due to the lesser concentration of enzymes in the appendices. It is, further, possible that the muscle sphincters at the caecal openings into the intestine were contracted, although no evidence of this was found in tissue sections.

After cutting the intestine a marked contrast is seen in wound healing between cortisone-treated and untreated trout. This is explained by the effect of the hormone on the connective tissue response to the trauma. The failure of the wound to close in the treated fish is correlated with the decrease in the fibrinous exudate. Spain *et al.* (1952) reported a drop in the level of circulating fibrinogen in cortisone-treated mice with turpentine-induced abscesses, and suggested that this lowered fibrinogen level was correlated with the scarcity of fibrin at the site of inflammation. Baker & Whitaker (1950) also noted delay in cutaneous wound closure in rats after topical application of adrenal cortical extract. These workers reported atrophy of collagen and impaired growth of granulation tissue with inhibition of fibroblast proliferation.

Inhibition of granulation tissue around turpentine abscesses after cortisone treatment has also been described by Shapiro and co-workers (1951), Taubenhaus *et al.* (1952) and Taubenhaus (1953). The effects of prolonged treatment of non-traumatized skin in rats given cortisone and compound F were noted by Castor & Baker (1950). Inhibition of fibroblast proliferation in rats following ACTH therapy was also reported by Baker & Ingle (1948). Similiar inhibition occurred in rats with turpentine abscesses after administration of testosterone propionate and estradiol dipropionate by Taubenhaus & Amromin (1949).

It therefore appears that cortisone, related steroids and ACTH retard wound healing by inhibition of connective tissue elements. It is also evident that the mechanism in the rainbow trout is similar to that reported for mammals.

Summary

1. Responses of digestive tract tissues were studied over a 72-hour period following turpentine injection and surgical trauma. Histological changes, characteristic of acute inflammation, were noted in the stomach, intestine and caeca one hour after injection, being most prominent in the cardiac stomach.
2. Increase in the number of fibroblasts and granule cells in the stomach was marked after 7 hours, followed by decrease in granule cell number at 24 hours with the appearance of "tubercles," returning to almost normal after 2 days. A cyclic activity of the granule cell is suggested.

3. Lesions in the intestine and caeca were less extensive; necrosis was attributed to pancreatic enzyme digestion.
4. Cortisone given concurrently with turpentine resulted in a delayed response, tissue removed after 24 hours resembling that seen 7 hours after turpentine alone. Following pretreatment with cortisone no lesions were apparent.
5. Increased phagocytosis coincident with inflammation was demonstrated after Thorotrast and turpentine injections. Augmentation of macrophagic activity was attributed to adrenal cortical stimulation of R-E elements with stress.
6. Cortisone injection preceding incising of the intestine resulted in inhibition of wound healing, with less granulation tissue, fewer inflammatory cells and more extensive necrosis.
7. The response of blood elements in the tissues corresponds with changes noted in the circulating leukocytes. The physiological responses in the rainbow trout are similiar to those reported in mammals under comparable conditions.

Literature Cited

Baker, B. L., & D. L. Ingle
1948. Growth inhibition in bone marrow following treatment with adrenocorticotropin (ACTH). Endocrinology, 43: 422-429.

Baker, B. L., & W. L. Whitaker
1950. Interference with wound healing by the local action of adrenocortical steroids. Endocrinology, 46: 544-551.

Cartwright, G. E., C. J. Grubler, L. D. Hamilton, N. M. Fellows & M. M. Wintrobe
1951. The role of the adrenal cortex in the regulation of the plasma iron level and the functional state of the reticulo-endothelial system. Proc. Second Clin. ACTH Conf., 1: 405-412. (J. R. Mote, Ed.) Blakiston, Philadelphia.

Castor, C. W., & B. L. Baker
1950. The local action of adrenocortical steroids on epidermis and connective tissue of the skin. Endocrinology, 47: 234-241.

Clark, E. R., & E. L. Clark
1920. Reactions of cells in the tail of amphibian larvae to injected croton oil (aseptic inflammation). Am. J. Anat., 27: 221-254.

Dougherty, T. F., & G. L. Schneebeli
1950. Role of Cortisone in regulation of inflammation. Proc. Soc. Exptl. Biol. Med., 75: 854-859.

Gordon, A. S., & G. F. Katsh
1949. The relation of the adrenal cortex to the structure and phagocytic activity of the macrophagic system. Ann. N. Y. Acad. Sci., 52: 1-30.

Menkin, V.
1933. A note on the mechanism of fixation in an area of sterile inflammation. Proc. Soc. Exptl. Biol. Med., 30: 1069-1076.
1940.1. Studies on inflammation. XVIII. On the mechanism of leucocytosis. Am. J. Pathol., 16: 13-32.
1940.2. Effect of adrenal cortex extract on capillary permeability. Am. J. Physiol., 129: 691-697.
1942. Further studies on effect of adrenal cortex extract and various steroids on capillary permeability. Proc. Soc. Exptl. Biol. Med., 51: 39-41.
1951.1. Effects of cortisone on the mechanism of increased capillary permeability to trypan blue in inflammation. Am. J. Physiol., 166: 509-517.
1951.2. Effects of ACTH on the mechanism of increased capillary permeability to trypan blue in inflammation. Am. J. Physiol., 166: 518-523.
1954. On the anti-inflammatory mechanism of hydrocortisone (compound F). Science, 120: 1026-1028.

Michael, M., Jr., & C. M. Whorton
1951. Delay of early inflammatory response by cortisone. Proc. Soc. Exptl. Biol. Med., 76: 754-756.

Moon, V. H., & G. A. Tershakovec
1951. The trigger mechanism of acute inflammation. Am. J. Pathol., 27: 693-695.

Rebuck, L. W., & R. C. Mellinger
1953. Interruption by topical cortisone of leucocytic cycle in acute inflammation in man. Ann. N. Y. Acad. Sci., 56: 715-732.

Robinson, H. J., & A. L. Smith
1953. The effect of adrenal cortical hormones on experimental infection. Ann. N. Y. Acad. Sci., 56: 757-763.

Shapiro, R., B. Taylor & M. Taubenhaus
1951. Local effects of cortisone on granulation tissue and the role of denervation and ischemia. Proc. Soc. Exptl. Biol. Med., 76: 854-857.

Spain, D. M., N. Molomut & A. Haber
1952. Studies of the cortisone effects on the inflammatory response. J. Lab. Clin. Med., 39: 383-389.

Taubenhaus, M.
1953. The influence of cortisone upon granula-

tion tissue and its synergism and antagonism to other hormones. Ann. N. Y. Acad. Sci., 56: 666-673.

Taubenhaus, M., & G. D. Amromin

1949. Influence of steroid hormones on granulation tissue. Endocrinology, 44: 359-367.

Taubenhaus, M., B. Taylor & J. V. Morton

1952. Hormonal interaction in the regulation of granulation tissue formation. Endocrinology, 51: 183-191.

Thomas, L.

1953. Cortisone and infection. Ann. N. Y. Acad. Sci., 56: 799-813.

Weinreb, E. L.

1958. Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri irideus*. I. Hematology: Under normal and experimental conditions of inflammation. Zoologica, 43: 145-154.

Weinreb, E. L., & N. M. Bilstad

1955. Histology of the digestive tract and adjacent structures of the rainbow trout, *Salmo gairdneri irideus*. Copeia, (3): 194-204.

Yokoyama, H. O.

1947. Studies on the origin, development, and seasonal variations in the blood cells of the perch, *Perca flavescens*. Unpublished Ph.D. thesis, University of Wisconsin, Madison.

EXPLANATION OF THE PLATES

The following figures were made from sections fixed in Helly's solution; all figures with the exception of Fig. 7, which was stained with PAS, were stained with hematoxylin-eosin-azure.

Plate I

Fig. 1. Cross-section of cardiac stomach 7 hours following turpentine injection, showing increase in granule cells (GC).

Fig. 2. Cross-section of cardiac stomach 24 hours following turpentine injection, showing inflammatory reaction in tunica propria and submucosa, with edema of the submucosa. Outlined area in submucosa encloses tubercle-like lesion shown at higher magnification in Fig. 3.

Fig. 3. High-power view of "tubercle," showing central necrosis, fusion of immature cells (I) and peripheral fibroblasts (F).

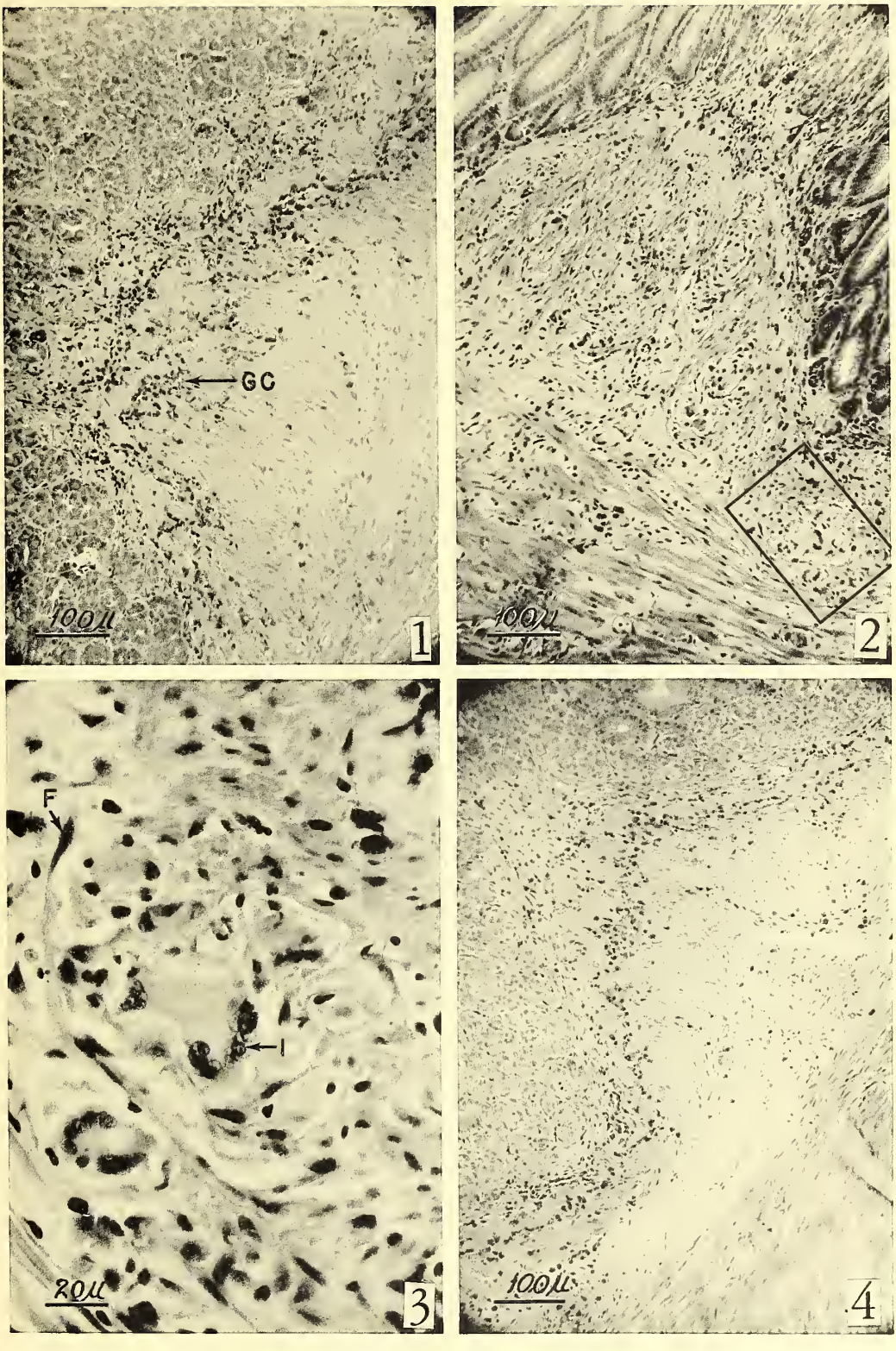
Fig. 4. Cross-section of cardiac stomach 24 hours following concurrent injections of turpentine and cortisone. Note lack of lesions and similarity to 7-hour reaction.

Plate II

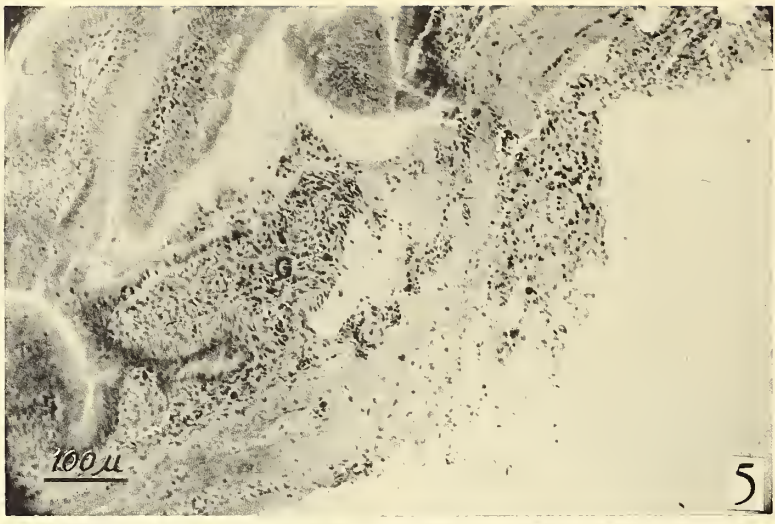
Fig. 5. Section of descending intestine in region of cut 24 hours following operation. Note inflammation, bridging of cut and granulation tissue (G).

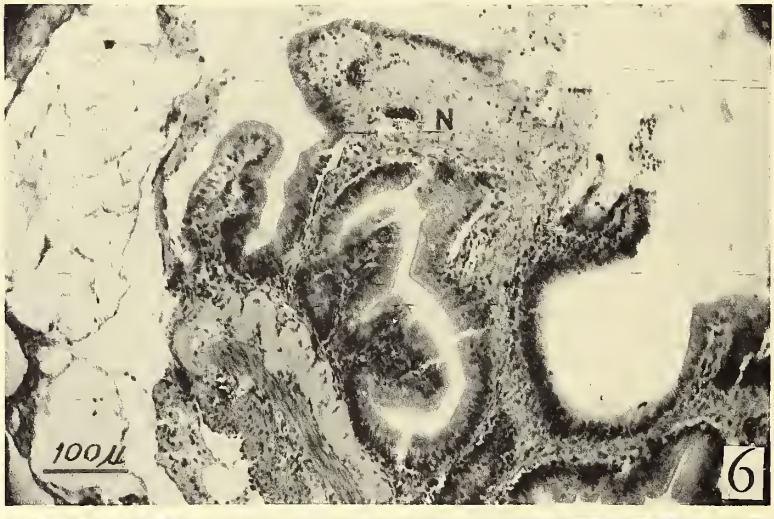
Fig. 6. Edge of cut in descending intestine 24 hours following operation and pretreatment with cortisone. Note less inflammatory response and necrosis (N).

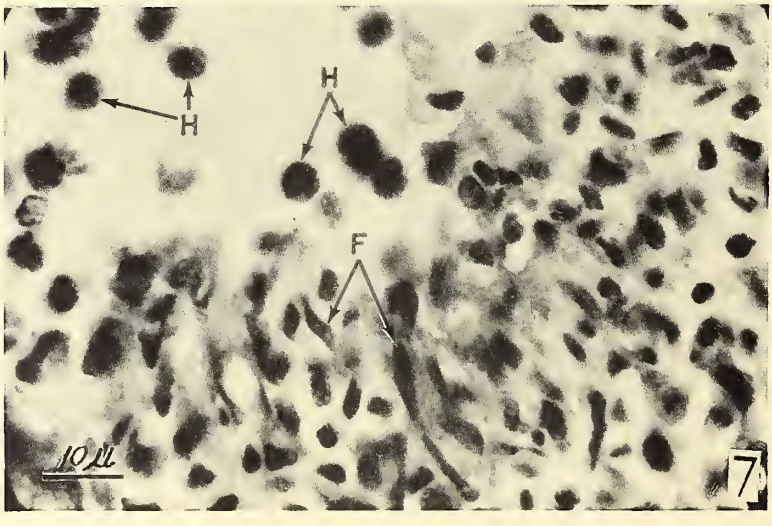
Fig. 7. Mucosa of descending intestine 72 hours following operation, showing cellular response to secondary infection. Heterophil, H; fibroblast, F.



STUDIES ON THE HISTOLOGY AND HISTOPATHOLOGY OF THE RAINBOW TROUT, SALMO GAIRDNERI IRIDEUS. PART II.







STUDIES ON THE HISTOLOGY AND HISTOPATHOLOGY OF THE RAINBOW TROUT, SALMO GAIRDNERI IRIDEUS. PART II.

3

A Study of the Structure and Development of Certain Reproductive Tissues of *Mugil cephalus* Linnaeus[1]

ALBERT H. STENGER

Department of Biology, Graduate School of Arts and Science, New York University, New York, N. Y.

(Plates I-VIII)

INTRODUCTION

ONE of the more common species of the South Atlantic and the Gulf of Mexico is *Mugil cephalus* Linnaeus, the black mullet, gray mullet or jumping mullet. It is also an important food fish of the warmer waters of China, Japan, India, Australia, South Africa, the Hawaiian Islands and the Mediterranean Sea. Because of its importance as a source of animal protein and its adaptability to pond culture, an increasing interest is being given to the propagation of this mullet abroad. Considerable investigation is being undertaken by government-sponsored projects on the mullet fishery, particularly in the Far East. In the United States, especially along the Gulf of Mexico coast where the mullet fisheries are of economic importance, a number of studies of the species have been published by state and national fishery bureaus. The State of Florida Board of Conservation and Miami University especially have been interested in various aspects of the Florida mullet fisheries.

The natural history of *Mugil cephalus* and fishery data are treated quite extensively in the following: Jacot (1920), Smith (1935), Kilby (1949), Kesteven (1942, 1953), Broadhead (1953), Thomson (1949, 1951, 1953), Gunter (1945), Roughley (1951), and Sarojini (1951). Little detailed, authentic information exists on the spawning habits of *Mugil cephalus*, however, and practically nothing is recorded on its embryology and organogenesis. Mullet eggs of various species have been described by Cunningham (1891-1892), Errenbaum (1909) and Sanzo (1936). The latter describes *M. cephalus* eggs (size, color, oil drop, etc.), fertilization, cleavage, hatching and the gross appearance of the young fry to the eighth day. The recent observations of Nair (1957) are similiar in scope and generally agree with Sanzo's 1936 report. No histological studies were made by either investigator. Dekhnik (1953) also describes the eggs of *M. cephalus* and three other species of Black Sea mullets. Jacot (1920) reports on the development of the young mullet in his study concerned with migration, scale annulation and the development of external features.[2]

Mugil cephalus appears to have no external sex markings or structures; sexing of the fish, except in the spawning season, is done by gross examination of the gonads. Broadhead (1953) has adapted the Australian scheme of sexing (Kesteven, 1942) to the Atlantic and Gulf of Mexico mullets. In the course of extensive experimentation on the pond cultivation of *M. cephalus* in brackish water at Marineland, Florida, Johnson (1954) found evidence to indicate that the sexing of this mullet, particularly the immature fish, was probably subject to some error. He also found some evidence– the presence of oocyte-like cells in young fish–that suggested

[1]Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Biology Department, New York University.

[2]Concerned with a closely related species of the Atlantic (*M. curema*) is the report of William W. Anderson (1957) on the "Early Development, Spawning, Growth, and Occurrence of the Silver Mullet (*Mugil curema*) along the South Atlantic Coast of the United States," U. S. Dept. Interior, Fish and Wildlife Service, Fish. Bull. No. 119, vol. 57: pp. 397-414. The author also reports in the same volume (Bull. 120, pp. 415-425) on "Larval Forms of the Fresh-water Mullet (*Agonostomus monticola*) from the open ocean off the Bahamas and South Atlantic Coast of the United States."

that the mullets might be hermaphroditic, possibly protandrous or protogynous. The literature discloses practially no information on the anatomy and histology of the reproductive system of the Mugilidae. Broadhead (1953) and others have commented on this lacuna. This study was undertaken to describe the anatomy and histology of the gonads of *Mugil cephalus*. In order to ascertain if some form of hermaphroditism existed in this mullet, a development study of the gonads from the earliest stage obtainable (20 mm. S. L. fry) to the mature fish was undertaken.

Thanks and acknowledgement are hereby tendered to Mr. Malcolm Johnson of the U. S. Department of Agriculture, formerly of the staff of the Marine Studios of Marineland, Florida, for the material and his generous cooperation. Also to Mr. F. G. Wood, Jr., Curator of the Marine Studios, who made the facilities of the Marineland Research Laboratory available during the summer of 1954. I am also indebted to Dr. William Tavolga of the American Museum of Natural History for many helpful suggestions and to Mr. Albert Bianchi for the translation of several Italian reports. Finally, I am grateful to Prof. Harry A. Charipper of New York University, whose sponsorship and cooperation made this study possible.

Materials and Methods

Tissues for histological examination were secured from fish cast-netted from the ocean and the Metanzas inland waterway, adjacent to the laboratory at Marineland, Florida.

Fish used in this study ranged in size from 19 mm. to 425 mm. Measurements of the large specimens were made on a standard board, and standard lengths were determined. Specimens under 100 mm. in length were measured after fixation and partial dehydration in 80% ethanol. Small fish were measured by means of a dissecting microscope. The gonads and adhering mesogonium were removed from the large fish immediately after death, washed for a few seconds in sea water to rid them of sand and grit, and stretched on strips of index card. After identification, they were placed in fixing solution. Small fish were anesthetized in 0.2% solution of Tricain methanesulfonate (MS-222) in sea water; the abdomen was then incised and the fish placed in the fixing solution.

Bouin's solution was used for the bulk of the material. Some tissues were fixed in 10% formalin in sea water; formol-fixed material was generally unsatisfactory since it exhibited relatively great shrinkage and poor staining quality.

After 24-hour fixation, the tissues were transferred to 70% ethanol and sent to New York. Here they were placed in 80% ethanol for at least 24 hours. By means of a dissecting microscope the small fish were measured and the fins and skin removed to facilitate sectioning. It was possible to remove the peritoneum with the adhering gonad from the 30 to 90 mm. specimens.

Dehydration was accomplished by the Zirkle Normal Butyl Alcohol series (Krajian, 1940, p. 212). Infiltration (two baths) and final embedding in fresh paraffin was in Fischer (56°-58°) Tissuemat. Routinely, sections were cut at 7 and 5 micra.

Two staining methods were utilized as routine procedure. The Alum Hematoxylin of Galigher (a modification of the Harris Hemalum) was used in conjunction with Triosin (0.5% in 90% ethanol), buffered with N/10 HCl (approx. pH 5.4), as recommended by Galigher (1934), gave nice differentiation of nuclei and cytoplasm. Some sections were stained with Lillie's Mayer Alum Hematoxylin, (Lillie, 1954, p. 76) and counterstained with Triosin. Some slides, in many instances alternate slides in a series, were stained with Masson's Ponceau-acid fuchsin, Anilin blue or light green technique (Lillie, 1954, p. 351). However, 1% phosphotungstic acid was substituted for phosphomolybdic acid. The Gomori elastin stain (Lillie, 1954, p. 364) was used to identify elastic connective tissue. The Krajian (1940, p. 111) version of the Foot reticulum stain proved satisfactory for demonstrating reticulum.

All stained sections were mounted in a solution of Clarite in Xylene.

Description

Morphology and Histology of the Indifferent Stages

20 mm. Fry, Collected Nov. 28-Dec. 24.—The gonad primordium of the 20 mm. fry consists of two microscopic fibers suspended from the pigmented peritoneum on either side of the dorsal mesentery. The strands form a "V" with its apex slightly anterior to the cloaca; each arm extends anteriorly the length of the abdominal cavity, *i.e.*, approximately 4 mm. The peritoneum with the attached genital primordium separates the abdominal cavity from the dorsal body cavity in which the swim bladder and mesonephros are developing (Figs. 1 and 1a). Transverse sections through the anterior abdominal region reveal the progonal masses as club- or leaf-shaped aggregates of mesenchymal cells enclosed in a reticulum-like capsule which is suspended from the peritoneum by a slender stalk or stem. The

non-germinal progonal areas contain developing nerves, blood vessels and varying amounts of black pigment. Sections through the converging posterior fibers show them to be essentially similar in shape. The capsule, however, is better developed, particularly the lateral and ventral borders, which are composed of squamous or low columar cells. This epithelium appears to be a proliferation from the peritoneum, via the stalk. At the extreme caudal end, the primordia appear as lateral outgrowths from the dorsal mesentery.

Germinal elements in the posterior primordia are represented by two structures: a cord of tissue which courses through the caudal third of each fiber, and germ cells which are embedded within the cords. In section, the cord is an irregularly shaped syncytium of finely granular, acidophilic material. Nuclei of the cord are round or ovoid and measure no more than 5 micra in diameter.

Germ cells (Fig. 2) lying in the ground cytoplasm of the syncytium are ovoid, relatively clear cells that measure 9 to 12 micra on their long diameter. Germ cell cytoplasm is faintly stippled with acidophilic material, and no basophilic or yolk granules are present. The nuclei are somewhat flattened spheres with their greatest diameter from 7 to 9 micra. The prominent nuclear membrane appears to be dotted with chromatin particles on its inner surface, and fine threads of chromatin anchored to the nuclear membrane form a network in the colorless karyoplasm. A conspicuously large, clear, faintly basophilic, eccentrically placed nucleolus is always present, and usually two or three smaller nucleoli are to be found in the chromatin mesh. An idiosome is usually discernable.

In two specimens in which counts could be made, it was found that one primordium contained 12 germ cells and the other 15. No germ cells were found outside of the gonadal anlage.

35-50 mm. Stage—Mullet fry collected in the latter part of February, in March and in the forepart of April measure from 35 to 50 mm. Sections of the gonad anlagen of these stages show the glands to be of similiar shape to the primordium of the younger fry, but considerably larger. Much of the increase in size is the result of the development of the non-germinal region which occupies the medial side. Sections from the anterior strands show vascular elements occupying most of the non-germinal region. The extreme caudal end of the strands appear as knobs on either side of the dorsal mesentery. Fig. 4, from a 50 mm. specimen, shows the knob-like appearance of the posterior region of the gonads.

Sections through the gonadal strands of a 40 mm. mullet, collected March 27, show the germinal portion of the gonad as an acidophilic band of tissue lying beneath the capsule. The band is similiar to the syncytial tissue described in the 20 mm. stage. A section from the mid-region of the gonad may show five or six germ cells imbedded in the syncytial band.

A 50 mm. specimen (Fig. 3) is essentially similiar in appearance. However, more germ cells are present; a 5-micra section contained 8 cells. Mitotic configurations in the germ cells are encountered with more frequency in this stage. The epithelial border is still generally limited to the lateral side and ventral end.

In specimens as small as 25 mm. there is some indication that cells from the lateral epithelial border of the gland are penetrating into the stroma at the point of juncture of the gland and the stalk. In the mid-region of the 40 mm. stage gonad, this proliferation is evidenced by a cord of flat cells. Subsequently the cord forms an epithelium-lined fissure which separates the gland into a lateral germinal region and a mesial mesogonium. Lateral outgrowths from the fissure form duct-like processes which extend to the germ cells on the periphery of the gland.

61 mm. Stage.—A 7-micra section through a gonad cord from a specimen collected on April 23 is shown in Fig. 5. The lighter-staining stroma of the gland appears to be divided by the darker-staining branches of the duct system. Peripheral germ cells have formed nests along the lateral and ventral borders (right and bottom) and some migration of germ cells in the ducts has taken place (lower right border).

75 mm. Stage.—Fig. 6 is from a section, approximately the mid-region, of a developing gonad from a 75 mm. fish, collected in August. The dark structure to the left of the gonad is the peritoneum. Nests of germ cells occupy most of the lateral edge and ventral end. Nests of germ cells are separated by septa of small cells which appear to be proliferations from the capsular epithelium of the gland. Note the hilus, indicated by an arrow, which appears between the body of the gonad and the stalk. At this point, epithelium from the stalk and peritoneum appears to be growing into the gland. From this ingrowth, a cord and subsequently a branched system of ducts form. The main duct more or less divides the primitive gonad into a lateral germinal region and a medial portion which is the future mesogonium. The latter is composed of developing connective tissue fibers. Note the large vascular elements in the mesogonial area (right). In time this region will also support nerves and small ganglia.

The germ nests along the lateral border of the gland are much larger than those seen heretofore. Some of the large nests, when followed through several sections, may contain as many as fifteen cells. Between the large germ cells are small irregularly shaped cells with sparse acidophilic cytoplasm. It appears that these elements are derived from the syncytial cord and probably from the capsular epithelium. Single germ cells and small groups of two or three cells, which apparently have broken away from the peripheral nests and have moved toward the center of the gland, are conspicuous in this stage.

100 mm. Stage.—Fig. 7 is from a larger specimen collected in February. The increase in the stromal tissue of the germinal and mesogonial regions is marked. Note the main duct which will become the vas deferens in the male.

Fig. 8 is from a section of a gonad of a 150 mm. specimen collected in May. The sex of the individual is not indicated by the structure of the gland. There are nests of germ cells and evidence of cord formation. If the nests form cords, maleness is indicated. The dark cell to the right of the figure and just below the center is one of the ova-like cells which are quite common in developing gonads. They appear to be small oocytes with normal-appearing nuclei. They have a large nucleolus and in some cases the chromatin threads can be seen. The cytoplasm, however, is abnormal. Frequently, the ground substance appears to be broken up and stains acidophilic rather than basophilic, as does the cytoplasm of normal oocytes—possibly an indication of degenerative changes.

The germ cells shown in Fig. 8 vary considerably in size and shape but are essentially similar to the much larger germ cells found in the peripheral nests of the young fry. The most prominent structure of germ cells is the nucleus, which occupies most of the cell. The nuclei always contain a large, eccentrically-placed, faintly basophilic, transparent nucleolus. In favorable sections, two or three small nucleoli can be seen and the chromatin threads are visible. The gonial cytoplasm contains varying amounts of faintly acidophilic particulate matter. With high magnifications, the idiosome may be seen.

The germ cells shown in the illustration do not represent all of the cells present in the area. With high magnification, many more single cells can be seen in the stroma and between the epithelial lining of the ducts and the stroma. Frequently, germ cells are flattened or crescentic in shape; however, their outline (cell membrane) is quite even. There appears to be no evidence that they assume amoeboid shape. This is in contrast to the cells of the ducts which are very irregular. At this stage there does not appear to be any indication of the sexual potentialities of germ cells.

Development of the Testes

Mullet testes from the August collection are slightly larger than those collected in early summer. Histologically, August glands might be characterized as representing a period of germ cell mitosis; only rarely is evidence of meiotic activity found. A rough measurement of the greatest diameter of a testis from a 225 mm. specimen collected in early August is less than 2 mm. The testis from a mullet of the same size, collected on October 28, measured 13 mm. in diameter. Histological examination shows that the October specimen is well on the way to ripeness.

Fig. 9 shows the general appearance of the maturing testes of a 250 mm. mullet collected in late summer. The stroma (the darker areas) consists of connective tissue. Selective staining shows the stroma is chiefly reticular (argyrophilic), collagenous and elastic fibers. A small, somewhat diffuse area of lymphoid-like tissue, appearing in six to eight different 7-micra sections, is usually encountered in the testes. There does not seem to be any uniformity in the size or position of lymphoid areas; they may be near the vas deferens or in the peripheral region. In some instances they appear to be separated from the surrounding tissue by a thin thread-like capsule; in other cases, the boundaries are diffuse.

The mesorchium of the mullet is made up of coarse collagenous fibers. It suspends the testes, supports the vas deferens and contains blood vessels, nerves and ganglia. In its caudal extremity the mesorchium becomes a heavy sheath that encloses the sperm duct which has formed by the fusion of the two vas deferentia. This anastomosis occurs a few millimeters from the cloaca.

The seminiferous tubules or sperm ducts, which are actually main and secondary branches of the vas deferens into which germ cells have lodged, project into the stroma. Fig. 10 shows the distal portions of the tubules from the August collection. The duct epithelial lining has been so disarranged by the plethora of germ cells as to have lost its morphological identity. The lumen of the tubules, frequently almost occluded by germ cells, becomes continuous with alveoli which develop in the peripheral germ cell nests.

The maturation of mullet male germ cells would appear to be similar to the pattern described in the perch by Turner (1919), and in *Cottus* by Hann (1927). After a period of cell

multiplication (August and September) the germ cells appear to shrink in size. This decrease seems to be caused by a diminution of the cytoplasm. The cytoplasm also stains with less intensity, although it is still acidophilic. Each small cell, presumably a spermatogonium, divides a number of times and forms an aggregate or cyst. Turner (1919) suggests that as many as six divisions occur in order to give rise to the number of spermatocytes in a cyst. This would seem to be true of the mullet. Turner (1919) and Hann (1927) report that each cyst has a fine membranous capsule. Hann finds the capsule somewhat difficult to resolve. This difficulty is also encountered in the mullet; a well-delineated and complete capsule does not seem to exist. From the incomplete capsules examined, the impression is formed that the capsule is composed of flattened duct epithelial cells. Fig. 11 shows a number of cysts in the tubules from the October collection. In some testes, degenerating cysts are numerous; they are compact aggregates of pycnotic nuclei in an acidophilic matrix. Turner (1919) finds degenerating spermatogonia in the perch.

The small size of the nuclei, and possibly unsatisfactory preservation, precludes a detailed description of maturation stages. Synaptene nuclei are evident but subsequent stages are impossible to identify. Young spermatids appear to be ovoid cells, less than 2 micra in length, with crescentic nuclei. Clusters of newly developed sperm appear in section as fan-shaped, or as Turner (1919) describes them, "parachutes". Fig. 13 contains several examples. It would seem that sperm in the "parachute" stage are not enclosed within a cyst; at least no membrane can be detected. Presumably the formation is maintained by the adhesion of the sperm tails. In ripe testes, spermatoza occur as dense, unorganized masses (Figs. 12 & 13) in the tubules and the vas deferens.

The Spent Testes

Fig. 14 from a 215 mm. fish is of an area from a spent testis. The general disarrangement of the tubules has resulted from a release of sperm and shrinkage of the organ. Under higher magnification one can identify small but typical germ cells in the walls of the tubule epithelium (circled area, Fig. 14). Nests of germ cells are on the periphery; many of the cells appear to be dividing. Fig. 15 shows a low power view of a section of spent testis from a larger specimen (275 mm.) The crenated edge of the gland is characteristic, The tubules are separated by coarse strands of connective tissue. There are fewer germ cells in the tubule epithelium of this specimen and the nests appear to be less in number and generally smaller. *i. e.*, they have fewer cells than the nests in Fig. 14. The paucity of germ cells suggests senescence.

In Fig. 14 the dark cells are two oocyte-like cells. Note that they have broken out of the nests. In Fig. 16, from the same specimen, is an oocyte-like cell in the vas deferens; note the sperm. This cell has the appearance of an immature, normal oocyte.

Development of the Ovary

The development of definitive ovaries in *Mugil cephalus* is indicated in fish measuring from 175 to 225 mm. standard length.

The gross macroscopic appearance of a developing ovary is similar to the immature testes, *i. e .*, two triangular-shaped strands of tissue suspended from the peritoneum anteriorly and the dorsal mesentery posteriorly (Fig. 17). With the increment and growth of oocytes the potential lobes of the ovary become cylindrical in shape and are enclosed in smooth muscle tunicae; positive identification is then possible.

Before gross changes in the presumptive ovary are discernable, it may be identified microscopically by the arrangement of the germ cells. Germ cells originating in the peripheral nests, which have migrated into the lumina of the ducts, undergo a number of divisions as undifferentiated germ cells, as in the developing testis. Ovarian differentiation is evidenced by the incorporation of a number of germ cells into a nest or nidus (Fig. 19). A nest may contain more than twenty cells of varying degrees of maturity. It is not unusual to find germ cells, oogonia and oocytes in the same nest. The transformation of germ cells into oocytes, like the formation of spermatocytes, is preceded by a diminution in the size of the germ cells followed by several mitotic divisions which form small oogonia. Young oocytes are made conspicuous by the basophilic ring of yolk material which forms around the nuclei.

A nest of germ cells, gonia, etc., is enclosed in a fine membranous capsule. The source of this capsule appears to be the duct epithelium and probably stromal cells. Within the nests are small, flat cells—similiar to duct epithelial cells —which become oriented around the oocytes. It is from these cells that the follicle cells develop.

Fig. 18 is from a caudal section of a 200 mm. length mullet, collected in February. The two lobes of the ovary are attached to the dorsal mesentery in this region. The mesovarium is much less developed than the mesorchium. Much of the stroma of the gland has been supplanted by the developing oocytes. Nests of oocytes have

broken down and finger-like lamellae of oocytes are forming. The fissure between the mesovarium and the glandular region (the vas deferens in the male) is becoming occluded by the increased volume of the ovary proper. Fig. 19 shows a small area from an anterior section of the same gland. The field is made up of maturing oocytes and some mature cells. The stroma of the ovary has been practically obliterated by the germ cells.

Fig. 20 shows the lamellae of a more mature ovary. The lamellae appear as hollow finger-like projections.

The development of the muscular tunica externa or capsule of the ovary proceeds in two stages. The thin serosa of the undifferentiated gland is overgrown by a pigmented connective tissue capsule which appears to originate in the stalk or presumptive mesovarium. Fig. 18 shows this overgrowth.

In the stage of development represented in Fig. 18, the tunica has an inner lining of squamous cells, an inner and an outer layer of collagenous and elastic connective tissue which run more or less longitudinally. Between the connective tissue layers are blood vessels, nerves and small ganglia. Islets of black pigment cells are enclosed in the connective tissue. The serosa of the gland is a pigmented layer which in places is several cells deep.

As development of the ovary proceeds, muscle fibers appear in the connective tissue capsule. They appear first near the mesovarium. In the near-ripe and ripe ovary the tunica is composed of two or three layers of smooth muscle which appear in transverse sections of the ovary to be running obliquely (Fig. 20). The ripe ovary does not appear to be pigmented and sections of the tunica do not show the pigmented serosa of the earlier stage. If a small piece of the tunica is examined with a dissecting microscope, the serosa is seen to contain regions of dispersed pigment cells.

The Ripe Ovary

Oocytes from ripening ovaries, collected in the early fall, show the coalescence of the fine, basophilic, cytoplasmic granular material into larger yolk granules. In the mature oocyte, a thin layer of the fine yolk precursor is retained beneath the cortex: this is shown in Fig. 23 as a dark layer beneath the zona radiata. Note also in Figs. 22 and 23 the clear round areas in the cytoplasm; presumably, they represent the spaces left by the dissolution of the oil. Sanzo (1936) reports that the eggs of *M. cephalus* have a diameter of 0.72 mm. and contain a polar oil drop 0.28 mm. in diameter.

The germinal vesicle occupies a central position in the oocyte; its membrane is wrinkled and uneven in contour. Nucleoli are arranged around the periphery of the vesicle—as many as fifteen may be seen in median, 10-micra sections. Lampbrush chromosomes are discernible in the finely stippled karyoplasm. Fig. 22 shows the peripheral regions of three oocytes. The conspicuous broad zona radiata has a typical radiate appearance. No vitelline membrane is detectable in the mullet oocyte. In sections stained with Gomori's elastin stain, the zona radiata appears to have a thin peripheral region which is stained by the fuchsin-aldehyde reagent.

The follicular epithelium of the mature oocyte is a membranous-like capsule of flat squamous cells (Fig. 22). Sections stained with reticulum stain show fine fibers surrounding the follicle.

The Spent Ovary

Fig. 23 is from a partially spent ovary collected in February from a fish of 225 mm. standard length. The lamellae are partially collapsed. The follicle cells of spent ovaries appear as strands and clumps of shrunken cells. The finer ovarian blood vessels also appear to be degenerating. Fig. 23 contains many atretic follicles; their ultimate course is not clear; presumably they are resorbed. Note also the small, dark young oocytes close to the lamellar membranes. Fig. 24, from a 275 mm. female, shows a small area from a spent ovarian lamella.

A Testis-Ovary

A single instance of hermaphroditism was found in the October 8 collection. The specimen was 250 mm. in length and by gross appearance of the gonads was sexed as a female. A section of one ovary is shown in Fig. 25. The other ovary contained fewer areas of male elements; in fact, many sections appear to be those from a normal ovary. At the top and to the right in Fig. 25 is a slit-like lumen. This appears to be a collapsed vas deferens; in normal ovaries this is not present. Most of the oocytes appear to be normal; however, small degenerating oocytes are numerous. In the figure the male region is most pronounced in the gray area to the left of center. This testicular area appears to be disorganized; if a tubular arrangement was present, it has been disrupted. Fig. 26 shows a small area from the male region. Note the normal appearance of the sperm parachutes and the several small oocytes. The latter appear abnormal although healthy-appearing oocytes can be found in close proximity to male elements. The suspensory tissue closely resembles a mesovarium and the muscular tunica is typically ovarian.

Discussion

Origin of Germ Cells

The literature on the ontogeny of vertebrate germ tissue reveals that during the past three-quarters of a century three general theories have been advanced to account for the origin of ova and sperm. The initial and oldest theory, that of the "germinal epithelium," which was originally proposed by Waldeyer in 1870 and according to Witschi (1948) abandoned by Waldeyer in 1906, proposed a sematic source of germinal elements. Nussbaum is generally credited with a second theory which proposed that germ cells are extra-embryonic in origin. Finally the idea has developed that extra-embryonic cells give rise to germ cells, but that they are supplanted or supplemented by germinal epithelium or other cells of somatic derivation. The often-quoted review by Heyes (1931) summarizes the literature pertaining to the origin of the vertebrate germ tissues to 1931. Everett (1945), Gillman (1948), Witschi (1948), Nieuwkoop (1949), Johnston (1951) and Nelson (1953) have reviewed the subject in the light of recent investigations.

Origin of Germ Cells in Fishes

Part of the literature dealing with the theories on the origin of germ cells and tissues in the vertebrates is concerned with the condition in fishes and indicates a similar division of opinion on the question of the genesis of germinal elements.

Followers of the original germinal epithelium theory of Waldeyer are in the minority; they include Hoffman (1886) and Bohi (1904). The second school of thought, which proposes a strict adherence to the Nussbaum theory, *i.e.*, a single source of germ cells, the primordial germ cells being of extra-embryonic origin, represents the majority of investigators, including Eigenmann (1891), Beard (1900), Allen (1911), Bachman (1914), Dodds (1910), Okelberg (1921), Hann (1927), Stromstein (1931), Johnston (1951) and Robertson (1953). Finally, there are those who admit the existence of primary germ cells, but who believe that sperm and ova arise from somatic cells as well. To this group of investigators belong Essenberg (1923), Foley (1927), Butcher (1929), Wolf (1931), Odum (1936) and Guerbilsky (1939).

Study of the development of the mullet gonad, from its appearance in the 20 mm. fry through maturity, indicates that the germ cells found in the 20 mm. stage are the antecedents of ova and sperm. As to the source of these primary germ cells, eggs or embryos not being available, one cannot say whether they are extra-embryonic, from an early cleavage stage as Eigenmann (1891) reported in *Micrometrus aggregatus*, or from the more common source, the gut-yolk sac endoderm; or whether they did not develop from peritoneal epithelium, as described by Hoffman (1886) in the salmon. If the large germ cells in the 20 mm. mullet fry are somatic, it might reasonably be expected that even in such a comparatively late stage there would be transitional stages between capsule or stromal cells and germ cells. Such was not the case.

Investigators generally have made a point of the fact that primary germ cells have no single morphological characteristic that makes them unique and identifiable.[3] They are usually referred to as large cells, sometimes as the largest cells in the embryo. Johnston (1951) gives a table of primordial germ cell dimensions, as reported in various forms. Comparison of Johnston's data and the reports of others indicates that the mullet germ cells, measuring 9 to 12 micra in diameter, are quite typical of the size of primary germ cells.

No yolk granules were seen in the germ cells of the mullet fry. The presence of yolk in the primary germ cells of vertebrates has been emphasized as a distinguishing characteristic by some authors, *e.g.*, Beard (1900) and Burger (1937). Generally, investigations on fish development bear this out. In some instances the germ cells retain some yolk after dividing in the gonocoel. On the other hand, Dodds (1910) found no yolk in the primary germ cells of *Lophius*, and Johnston (1951) reports the same condition in the freshwater bass. Both investigators traced the germ cells back to a premigration period.

Johnston (1951) states that he was able to differentiate primary germ cells from blood cells which they are said to resemble. Jordan (1917) and Risley (1933) stress the similarity of germ cells and blood cells. In the mullet, the extreme dissimilarity in size of the two types of cells gave no cause for confusion.

Germ Cell Nuclei and Nucleoli

Lobed nuclei are not characteristic of the mullet germ cells, as reported in *Raja* by Beard (1902), in the toadfish by Sink (1912) and in the guppy by Goodrich, Dee, Flynn & Mercer (1934).

---

[3]McKay, Hertzig, Adams & Danziger (1953) report that human primordial germ cells are positive to the alkaline phosphatase reaction. Subsequently, Chiquoine & Rothenburg (1957) have found that the primordial germ cells of the chick and *Ambystoma* are negative to this test. These reports suggest the possibility of phylogenetic differences in the phosphatase characteristics and further investigation of this subject seems warranted.

Most descriptions of germ cells note the presence of two or three nucleoli, as in the mullet. Dodds (1910) describes the extrusion of nucleoli in the early germ cells. He suggests that this phenomenon is a unique characteristic of germ cells. No evidence of nucleolar extrusion was seen in the germ cells of *M. cephalus.*

The Genital Strand

The position and general morphology of the genital strands in the young mullet are essentially the same as has been described in other fishes. However, the strands in mullet fry appear to have developed independently of the presence of germ cells. These strands are reported to be interrupted in the lamprey by Okelberg (1921) and in the bass by Johnston (1951). The latter compares the early gonad of the bass to a string of beads. Each bead might be said to represent a local proliferation of the peritoneal epithelium which has been evoked by the primordial germ cells. In the mullet, as in Fundulus (Bachman, 1914), in the toadfish (Odum, 1936), and in some other forms, the genital strand is not confined to areas which contain germ cells. From this fact and from the presence of a progonal region which is the supporting structure for nerves and blood vessels, one might infer that the strand did not develop in response to the presence of germ cells. Burns (1955) cites several studies, principally based on amphibian experimentation, which indicate that germ cells alone cannot induce the genital ridge and are not essential for its origin.

Maturescence of the Testes

The inception of maturescence in the gonads of *Mugil cephalus* is essentially the proliferation of germ cells from the peripheral nests toward the center of the gland. In the developing testes this proliferation contrives to form the tubules of germ cells within the walls of the duct system. This is the pattern reported for a number of forms, *e.g.,* Turner (1919) in the perch; Hann (1927) for *Cottus;* Stromstein (1931) in the goldfish; Goodrich, Dee, Flynn & Mercer (1934) in the guppy; Wolf (1931) in the platyfish; Jones (1940) in the salmon; and Johnston (1951) in the largemouth bass. Descriptions of testes in a number of other fishes indicate a similar architecture – stickleback, Craig-Bennett (1930); *Betta splendens,* Bennington (1936); largemouth bass and bluegill, James (1946a); goldfish, Kinoshita (1933); *Fundulus,* Mathews (1938), salmon, Weisel (1943) and Jones (1940).

Turner (1919) notes that the tubules (he refers to them as lobules) resemble the mammalian seminiferous tubules. Stromstein (1931) refers to them as seminiferous tubules. Bullough (1939) and Craig-Bennett (1930) state that they are not true seminiferous tubules. Craig-Bennett (1930) finds that the tubules of the stickleback contain no permanent germinal epithelium. In the mullet, residual germ cells are present in the post-spawned tubules and in the peripheral nests. Hann (1919) notes a similar condition in *Cottus* and Bennington (1936) in *Betta splendens.*

Turner (1919) and Foley (1927) report that male germ cells have their origin outside the testes and Geiser (1922) mentions "inconspicuous germ cells" migrating into the lobules from the soma of *Gambusia.* Regarding the source of the perch male germ cells, Turner (1919) states (p. 692): "A cord of germ cells outside the testes was found in a single specimen which was killed on May 5. Unfortunately, this was the only fish taken at this date and, though the cord has been sought in specimens taken at other dates, it has not been found." Foley (1927) believes that the male germ cells of *Umbra lima* come from stromal cells. Johnston (1951) on the largemouth bass, Wolf (1931) and Chavin & Gordon (1951) on the platyfish, and Essenberg (1923) on the swordtail depict a development of the testes essentially the same as that shown in the mullet. Essenberg finds, however, that some of the peripheral nests of germ cells (he refers to them as "primordial germ cells") degenerate and the fate of the others is uncertain. The duct epithelial cells divide and form spermatocytes, spermatids, etc., while the spermatocysts form within the ducts or tubules. Wolf (1931) disagrees with Essenberg's thesis; he reports that in the swordtail and platyfish, sperm are descendants of primordial germ cells and that duct epithelium does not form germ cells. Chavin & Gordon (1951) subscribe to this observation. Friess (1933) finds that duct epithelium of the swordtail is secretory; furthermore, she doubts if duct epithelium ever transforms into germinal cells. Goodrich, Dee, Flynn & Mercer (1934) state that the development of the testes in *Lebistes* is similiar to Wolf's description in the platyfish. Lavenda (1949), reporting on the protogynous hermaphroditism of the Atlantic sea bass. *Centropristes striatus,* reports that the testicular germ cells develop from the epithelial cells of the oviduct.

At one period during the early stages of the present study, before a complete series of specimens was examined, it appeared that the duct epithelium might be transforming into germ cells. Duct cells do increase in size and number

during growth and presence of germ cells along the base of the duct epithelium suggested such a transformation. Later, however, when a complete developmental series was examined, the independence of the two systems became obvious.

The Interstitium

The presence of endocrine interstitial cells has not been demonstrated in all fishes, either morphologically or physiologically. Courrier (1921) and Craig-Bennett (1930) state that the lymphoid tissue of the stickleback kidney and the endocrine interstitium of the testis are similiar in appearance. The lymphoid-appearing tissue of the mullet testis was examined carefully; no changes could be detected in the cytology of the elements during the maturation period. Possibly, as Craig-Bennett (1930) believes, osmic fixation is preferable to Bouin's fluid for demonstrating the granules, etc. James (1946b) describes clusters of cells in the interlobular tissue of largemouth bass testis, which she believes are glandular interstitial cells. Mathews (1938) reports "peculiar cells" in the testis of *Fundulus* which shrink after spawning. He interprets these cells as endocrine cells.

The interstitium of the mullet testis contained no cells which resemble the interstitial Leydig cells of common laboratory mammals.

Recently, Marshall & Lofts (1956) and Lofts & Marshall (1957) have reported that two distinct arrangements of the testicular endocrine cells occur in fishes. In the pike, char, *Labeo* and probably in some salmon, the glandular interstitium is in the lobules (tubules). The other type, reminiscent of the mammalian Leydig cells in which the endocrine interstitium rests between the tubules, is represented by the stickleback, sprat, *Tilapia* and some elasmobranchs. Marshall & Lofts (1956) report that the Leydig cells in both types are at first sudanophilic and then become cholesterol-positive after spawning.

Maturescence of the Ovary

The oocytes of *Mugil cephalus* are derived solely from germ cells; neither peritoneal (duct epithelium) nor stroma cells contribute to their numbers. Okelberg (1921), Hann (1917), Johnston (1951) and others have indicated that primordial germ cells are the sole progenitors of oocytes. Essenberg (1923) reports a unique condition to the swordtail. He believes that the primordial germ cells degenerate in potential female swordtails and that the functional oocytes are derived from peritoneal cells. Friess (1933) doubts that a somatic origin of oocytes exists in the swordtail and suggests that the marked degeneration reported by Essenberg is actually maturation. Wolf (1931) states that in *Platypoecilus maculatus,* oocytes have a duel origin; the greater number are derived from germ cells and the rest from somatic cells or perhaps from peritoneal cells. He reports some degeneration of germ cells, but not to the extent mentioned by Essenberg (1923) in the swordtail. Johnston (1951) has questioned the idea of a dual origin of oocyte and sperm. The point is well-taken. However, as Wolf (1931) intimates and as the studies on the mammalian ovary bear out (see references listed in the discussion of the origin of verebrate germ cells), evidence based on histological obesrvations has limitations, both technical and interpretative.

Goodrich, Dee, Flynn & Mercer (1934) point out the similarity of the development of the guppy and the platyfish and agree with Wolf (1931) that oocytes may arise from stroma cells, but that sperm originate only with germ cells. Dildine (1937), reporting on hermaphroditism in Lebistes, a phenomenon not mentioned by Goodrich, Dee, Flynn & Mercer (1934), did not find a transition of somatic cells to sperm or ova. Odum (1936) states that in *Opsanus tau,* oocytes develop from somatic cells as also does Guerbilsky (1939) in the mirror carp. On the other hand, Moore (1937), reporting on the rainbow trout, Robertson (1953) on the salmon, and Stromstein (1931) on the goldfish, declare that only germ cells give rise to oocytes.

Source of New Oocytes

Almost as controversial as the question of the origin of germ oocytes and sperm are the ideas on the source of new oocytes in the spent ovary. In the mullet, new oocytes are derived from the residual oogonia and germ cells in the periphery of the lamellae. Wheeler (1924), referring to the dab, Bullough (1939) to the European minnow and Craig-Bennett (1930) to the stickleback, report that some of the new oocytes are derived from the follicle cells of extruded oocytes. The concensus of those reporting on fishes appears to be that oogonia and young oocytes only form the new crop of oocytes and that the follicle cells degenerate.

Vitelline Body

Okelburg (1921) reports the presence of a vitelline body in the oocytes of the lamprey. James (1946a) mentions the presence of a yolk nucleus in the largemouth bass. Presumably, these structures are the same as the idiosome. The latter was visible in the oocytes until the time of the formation of the yolk particles.

Extrusion of Nucleoli

Essenberg (1923) and Stromstein (1931), reporting on the swordtail and goldfish, have described extensive extrusive nucleoli of oocytes. Essenberg states that the nucleoli of the helleri oocyte and indifferent germ cells pass into the cytoplasm and thence through the cell membrane into the follicle. As many as six nucleoli are seen in an oocyte. In the goldfish, Stromstein notes that nucleoli bud and divide into cytoplasm. He says (1931, p. 10): "This would seem to indicate that nucleolar extrusions are taking place at this time and that they are moving through the cytoplasm toward the periphery of the cells where a little later they form a definite zone." Comparable phenomena have not been observed in mullet oocytes. Oka (1931) believes that the nucleolus of the early normal *Oryzias* oocyte is replaced by scattered nucleoli, while in the "pseudocytes" (oocyte-like cells of males), the single nucleolus remains until a late stage. It has been impossible to confirm this nucleolar difference in the mullet.

Corpus Luteum

The literature contains reports on the appearance of corpus luteum-like structures in both live-bearing and egg-laying fishes. Samuel (1943) describes the development of structures in the elasmobranch, *Rhinobatus granulatus,* which are thought to be corpora lutea. Mathews (1938) believes that corpora lutea are present in the post-ovulatory follicle of *Fundulus heteroclitus.* Bretschneider & Duyvené de Wit (1947) also report corpora to be present in a number of teleosts and Selachii, describing the corpora as pre-ovulatory. Among the forms mentioned by these authors as possessing corpora is *Xiphophorus.* Friess (1933) describes "rest Korper" in the ovary and viscera of *X. helleri.* She believes that these bodies are formed from degenerate oocytes which have been invaded by special stromal cells, "Wanderzellen." She proposes that rest bodies may be involved in tumor formation. Chidester (1917) also found ovarian tissue scattered throughout the body of *Fundulus.*

Pickford & Atz (1957) have examined the evidence for the presence of corpora lutea in fishes; they state that no direct evidence has yet been submitted to show that the so-called corpora described by various investigators have a secretory function.

Fig. 23 from a partially spent *M. cephalus* ovary contains a great number of degenerating oocytes in varying degrees of atresia. Neither in this specimen nor in others was there any structure which bore any resemblance to the corpora lutea of mammals or to those reported in fishes.

Hermaphroditism

Numerous descriptions of hermaphrodite fish are encountered in the literature. Frequently, the instances of this phenomenon are reported as oddities or abberations. Thus the term "hermaphrodite" has taken on somewhat broad interpretations. In its strictest sense it describes the condition, as found in certain basses, wherein the gonad has a male and a female lobe or region which appear functional, possibly to the extent of being capable of self-fertilization. Hermaphroditism may also mean the quite common occurrence of varying numbers of oocyte-like elements in the embryo, the immature fish, or in the adult male. Finally, the term may describe sex reversal, protogyny or protandry.

Brock (1878) quotes Cavolini (1792) and others to the effect that certain of the sea basses are capable of self-fertilization. This claim has not been confirmed. However, the basses and related fishes have been shown to be unusual in their sex patterns. Van Oordt (1929) investigated *Serranus* and *Sargus,* and D'Ancona (1950) studied a number of serranids, sparids and related forms. Some of these fishes are stated to be functional hermaphrodites. D'Ancona (1950, P. 283) says: "J'ai pu constater chez *Serranus scriba* et *Hepatus hepatus,* la présence d'un hermaphrodisme functional constant . . ." Longley & Hildebrand (1940) report that certain basses from the Tortugas, Florida, region are functional hermaphrodites.

Cases of hermaphroditism in the embryonic or immature stages of fish, frequently manifested by the presence of oocyte-like cells in developing males, have been reported in the following forms: *Myxine glutinosa* (Cunningham, 1891-92 and 1886-87), Serranidae, Sparidae and the European eel (D'Ancona, 1945 and 1950), *Lebistes reticulatus* (Dildine[4], 1936), lamprey, *Entosphenus wilderi* (Okelberg, 1921), goldfish (Stromstein, 1931) and *Xiphophorus helleri* (Friess, 1933).

In the following species, hermaphroditism has been reported in the adult form (here again, the incidence of oocyte-like elements occurs in males): *Lepidosiren paradox,* (Agar, 1910), *Fundulus heteroclitus* (Chidester, 1917), *Bdellostoma* and *Myxine* (Cole, 1905, and Conel, 1917), trout (De Beer, 1924), *Phoxinus laevis* (Bullough, 1939), *Abramis brama* (Gryazeva, 1936), largemouth bass (James, 1946), *Carassius auratus* and *Sparus longispinis* (Kinoshita,

[4]Vaupel (1929), Goodrich, Dee, Flynn & Mercer (1934)and Berkowitz (1938) mention no hermaphroditism in the guppy.

1933 and 1936), *Fundulus majalis* (Newman, 1908), perch (Turner, 1928), *Oryzias latipes* (Oka, 1931), sea bream (Aoyama, 1955), *Serranus* and *Sargus* (van Oordt, 1929, and D'Ancona, 1945 and 1950). It should be noted that oocyte-like cells have been elicited experimentally by hormone and transplantation techniques (see Pickford & Atz, 1957).

Sex Anomalies in M. cephalus

Information on hermaphroditism in *Mugil cephalus* appears to be limited to a statement by Kesteven (1942) that "hermaphrodite roe are not very rare, one or two being found each season." Presumably these instances were based upon the gross examination of the gland. The present study demonstrates that anomalies in the gonads may not be detected by gross inspection. The presence of oocyte-like elements in immature glands or in testes are disclosed only in sectioned material. The hermaphrodite gland (Fig. 25) is a case in point. This ovary appeared to be quite normal; sections, however, revealed the presence of male elements.

Oocyte-like Cells.— In the mullet, oocyte-like cells appear in the undifferentiated gonad in specimens as small as 100 mm. Oocyte-like elements in the young fish never reach the size of those found in the mature males. They degenerate before the gland matures. As tube formation progresses in the males, oocyte-like cells continue to form. In the near-ripe, ripe, and spent testes, the oocyte-like cells are larger and more closely resemble true oocytes. The cytoplasm has the same basophilia and fine granular appearance as true oocytes. Whether this basophilia represents yolk precursor has not been determined. The other elements of the cell (nuclear membrane, nucleoli, lampbrush chromatin) are similar to young oocytes. Oka (1931) reports that the oocyte-like cells which he calls "pseudocytes" were restricted, in two *Oryzias* males, to a definite region of the testes and in a third specimen were distributed throughout the gland (as in the mullet). Oka (1931) finds that the "pseudocytes" develop in the same cyst with sperm. Agar (1910) also found this to be true of the lungfish. It appears to be true of the mullet.

D'Ancona (1945) states that oocyte-like cells of the eel and some other higher vertebrates are the result of an abbreviated oogenesis (oogénèse abrégée) in which such cells are formed directly from "protogonia" (primary germ cells) without the usual sequence of oogonia, etc. Observations on the mullet give some credence to this theory. In several specimens approximating 110 mm. in length, it appeared that the oocyte-like cells were most frequently seen in the peripheral nests. This might suggest that certain of the nest cells matured (?) precociously. As to why such cells should become feminized remains unanswered.

Okelberg (1921) reports in the lamprey and Grassi (1919) and D'Ancona (1950) in the eel that all gonads contain oocyte-like cells. Okelberg believes that the young lamprey is bisexual. Germ cells that show a tendency toward rapid division and formation of cysts are potentially male, whereas those that exhibit rapid growth are female. If both tendencies are equal, a temporary hermaphroditism occurs. When an imbalance in maleness or femaleness develops, the gonad becomes either male or female. When sex is finally established, the opposite sex elements gradually disappear. The undeveloped ova (oocytes) found in males represent the residual female elements of the bisexual stage. The condition in the eel is comparable (D'Ancona, 1950). Essentially this appears to be the pattern found in some other vertebrates, *e.g., Rana sylvatica, Sternotherus odoratus* and *Bufo lentiginosus*, as reported by Witschi (1921), Risley (1933) and King (1910). No adequate explanation for this seeming proclivity for femaleness in certain vertebrates has been offered.

Van Oordt (1929), Kinoshita (1936) and Lavenda (1949) have reported sex reversal (protogyny or protandry) as occurring in the sea basses and some related groups. Liu (1944) states that the symbranchid eel, *Monopterus javanensis,* is protogynous. All young fish are females and breed as such. Male germ cells develop in the ovary and eventually all females become functional males. Zwei (1950) describes three Mediterranean fish, *Maena samaris, M. chryselis* and *Paegellus erythrinus,* which are also protogynous. Regarding sex reversal in poeciliid fishes, Gordon (quoted by Pickford & Atz, 1957, p. 196) believes that Essenberg's (1926) claim of two cases of protogyny in *X. helleri* is open to some doubt, principally because no other examples of sex reversal have appeared in perhaps hundreds of masculinized swordtails that have been observed before and after 1926. Tavolga (1949) states that only two cases of sex reversal are recorded for the closely related platyfish.

Evidence from the present study does not indicate that the mullet is protogynous or protandrous. In the two examples of protogyny cited, the observers note that mature females are smaller than males. In the mullet, the opposite appears to be true. Several males from the May collection, measuring 175 mm., had residual sperm in the vas deferens. Females from the

February collection which measured 200 to 225 mm. were developing definite ovaries. The material used in this study suggests that normally females under 250 mm. in length do not spawn. A single ripening female in the October 28 collection measured only 150 mm. Mr. Malcolm Johnson, who made the collection, tells me that of the hundreds of mullets which he examined, this was the smallest ripening female seen.

Other investigators have suggested that *Mugil cephalus* males mature at a smaller size than females. Hotta (1955), reporting on *Mugil japonicus* (which, the author states, is believed to be identical with *Mugil cephalus*), finds that mature females have a body length greater than 45 cm. and that males are less than 40 cm. in length. Thomson (1951) examined 135 male and 92 female mullets more than 25 cm. in length, from Western Australian waters. No spent gonads were found in males smaller than 32 cm. or in females less than 35 cm. Broadhead (1953) reports that in the Gulf of Mexico, female mullets are larger than males. Of the mullets of the Salton Sea, California, Dill (1944) says that in a sample of large mullets, males averaged 16.5 in. (412 mm.) in length and females averaged 22.5 in. (562 mm.). Breder (1940), in describing the mating behavior of a group of mullets in a Florida creek, says that the males were about two-thirds the length of the females. Kesteven (1942) and others have suggested that the mullet male matures at an earlier stage than does the female. The histological evidence in the present study suggests that male mullets breed at the end of their second year. Females appear to be a year older. Bromhall (1954), speaking of the Hongkong mullets (*M. cephalus*), says that there is a primary and secondary spawning and he believes that the spawnings have a lunar periodicity. There seems to be some evidence to suggest that Florida mullets spawn more than once in a season. A number of females and males showed partially spawned gonads.

Conclusions

1. The germinal tissues of *Mugil cephalus* L. are derived from large germ cells which are found in the gonad anlage of the 20 mm. fry. The role of these cells in the formation of sperm and oocytes and their similarity to the primordial germ cells of other fishes suggests that they are primordial germ cells.
2. No evidence has been found to support the belief that peritoneal or other somatic cells transform into gametic elements.
3. Mullets exhibit a juvenile, sexually-indifferent stage.
4. Oocyte-like germ cells are found in varying amounts in the indifferent gonads and in the testes of mature and spent males.
5. The presence of oocyte-like cells in immature and mature male mullets suggests intersexuality rather than protogyny.
6. The gonad of a unique, hermaphrodite, mature mullet is described.
7. The evidence from this study suggests that male mullets mature sexually at a smaller size than do females. Males, it would appear, become mature at about two years of age; females first ripen during their third year.

Bibliography

Agar, W. E.
1912. The spermatogenesis of *Lepidosiren paradox*. Quart. J. Micr. Sci., 57: 1-44.

Allen, B. E.
1911. The origin of the sex-cells of Amia and Lepidosteus. J. Morph., 22: 1-36.

Aoyama, Tsuneo
1955. On the hermaphroditism in the Yellow Sea Bream, *Taius tumifrons*. Jap. Journ. Ichthyol., 4: 119-129.

Bachman, Freda M.
1914. The Migration of the germ cells in *Ameiurus nebulosus*. Biol. Bull., 26: 351-367.

Beard, John
1900. The morphological continuity of the germ-cells in *Raja batis*. Anat. Anz., 18: 465-478.
1902. The germ cells (Part I *Raja batis*). Zool. Jahrb., 16: 615-702.

Bennington, N. L.
1936. Germ cell origin and spermatogenesis in the Siamese Fighting Fish, *Betta splendens*. J. Morph., 60: 103-125.

Berkowitz, Philip
1938. The effects of estrogenic substances in *Lebistes reticulatus* (Guppy). Anat. Rec., 71: 161-169.

Bohi, U.
1904. Beitrage zur Entwicklungsgeschichte der Lebenshohle und der ersten Genitalanlage bei den Salmonoiden. Morph. Jahrb., 32: 505-586.

Breder, Jr., C. M.
1940. The spawning of *Mugil cephalus* on the Florida West Coast. Copeia, 1940, #2: 138-139.

Bretschneider, L. H., & J. J. Duyvené de Wit
1947. Sexual Endocrinology of Non-mammalian

Vertebrates. Elsevier Publ. Co., Inc., New York.

Broadhead, Gordon C.
1953. Investigation of the Black Mullet, *Mugil cephalus* L. in Northwest Florida. State of Florida Board of Conservation Techn., Series No. 7, Univ. of Miami, Florida.

Brock, J.
1878. Beitrage zur Anatomie und Histologie der Geschlechtsorgane der Knochenfische. Morph. Jahrb., 4.

Bromhall, J. D.
1954. A note on the reproduction of the grey mullet, *Mugil cephalus*. Honkong Univ., Fish Journ., #1, Dec., 1954.

Bullough, W. S.
1939. A study of the reproductive cycle of the minnow in relation to the environment. Proc. Biol. Soc., Lond., 109: 79-102.

Burger, J. W.
1937. The continuity of the germ cells in the Urodele, *Plethodon cinereus* (Green). J. Morph., 60: 489-519.

Burns, R. K.
1955. Chapter 6 in "Analysis of Development." W. B. Saunders Co., Philadelphia and London.

Butcher, E. O.
1929. The origin of the germ cells in the Lake Lamprey (*Petromyzon marinus unicolor*). Biol. Bull., 56: 87-98.

Cavolini, P.
1792. Ueber die Erzeugung der Fische und Krebse. Quoted by Brock (1878) and Lavenda (1949).

Chavin, Walter, & Myron Gordon
1951. Sex determination in *Platypoecilus maculatus*. Zoologica, 36: 135-145.

Chidester, F. E.
1917. Hermaphroditism in *Fundulus heteroclitus*. Anat. Rec., 12: 389-396.

Chiquoine, A. Duncan, & Edgar J. Rothenberg
1957. A note on alkaline phosphatase activity of germ cells in Ambystoma and chick embryos. Anat. Rec., 127: 31-35.

Cole, F. J.
1905. Note on Myxine. Anat. Anz., 27: 324.

Conel, J. L.
1917. The urogenital system of Myxinoids. J. Morph., 29: 75-163.

Courrier, R.
1921. Sur l'existence d'une glande interstitielle dans la testicules des poissons. Comp. Rend. Soc. Biol., 85: 939-941.

Craig-Bennett, A.
1930. The reproductive cycle of the three-spined stickleback, *Gasterosteus aculeatus*, Linn. Phil. Trans., Series B, 219: 197-280.

Cunningham, J. T.
1886-87. On the structure and development of the reproductive elements in *Myxine glutinosa*, L. Quart. J. Micr. Sci., 27: 49-75.
1891-92. On some larval stages of fishes. J. Mar. Bio. Assn. U. K., 2: 73.

D'Ancona, Umberto
1945. Sexual differentiation of the gonad and the sexualization of the germ cells in Teleosts. Nature, 156: 603-604.
1950. Determination et Differentiation du Sexe chez les Poissons. Archives d'Anatomie microscopique et de Morphologie experimentale, 39: 274-294.

De Beer, G. R.
1924. Notes on a hermaphrodite trout. Anat. Rec., 27: 61-62.

Dekhnik, T. V.
1953. Reproduction of *Mugil cephalus* in the Black Sea. C. R. Acad. Sci., U.R.S.S., n.s. 93: 201-204.

Dildine, G. C.
1936. Studies in teleostean reproduction. I. Embryonic hermaphroditism in *Lebistes reticulatus*. J. Morph., 60: 261-277.

Dill, Wm. A.
1944. The fishery of the lower Colorado River, Calif. Fish and Game, 30, #3: 163-167.

Dodds, G.
1910. The germ cells of Lophius. J. Morph., 21: 563-595.

Eigenmann, C. H.
1891. On the precocious segregation of the sex cells in *Micrometrus aggregatus*. J. Morph., 5: 481-492.

Errenbaum, E.
1909. Eier und Larven von Fischen. Nordische Plankton, Zoologischer Teil, #1: 132-133.

Essenberg, J. M.
1923. Sex differentiation in the viviparous Teleost *Xiphophorus helleri* Haeckel. Bio. Bull., 45: 46-96.
1926. Complete sex reversal in the viviparous teleost *Xiphophorus helleri*. Bio. Bull., 51: 98-111.

Everett, N. B.
1945. The present status of the germ cell problem in vertebrates. Biol. Rev., 20: 45-55.

Foley, James O.
1927. Observations on the germ cell origin in the adult male teleost, *Umbra limi*. Anat. Rec., 35: 379-399.

Friess, Else
1933. Untersuchungen uber die Geschlechtsumkehr bei *Xiphophorus helleri* Heckel. Archiv. fur Entwicklungsmechanik der Organismen, 129: 255-355.

Galigher, Albert
1934. The Essentials of Practical Microtechnique. Albert Galigher, Inc., Berkeley, Calif.

Geiser, S. W.
1922. Seasonal changes in the testes of *Gambusia affinis*, the top-minnow. Anat. Rec., 23: 104-105. Abstr.

Gerbil'skii, N. L., see Guerbilsky, N. L.

Gillman, Joseph
1948. The development of the gonads in man, with a consideration of the role of fetal endocrines and the histogenesis of ovarian tumors. Carnegie Inst. Wash., Contrib. to Embryol., 210: 81-131.

Goodrich, H. B., J. E. Dee, C. M. Flynn & R. N. Mercer
1934. Germ cells and sex differentiation in *Lebistes reticulatus*. Biol. Bull., 67: 83-96.

Grassi, B.
1919. Nuove ricerche sulla storia naturale dell' Anguilla. R. Com. Talass. Ital., Mem. 67.

Gryazeva, E. D.
1936. On the alteration of gonads of *Abramis brama* as connected with the ripening of generative products and age. Bull. Inst. Rech. Biol. Perm., 10: 285-384.

Guerbilsky, N. L.
1939. The development of the mirror-carp's ovocytes and its dependence on temperature. Arch. Russ. Anat. Hist. Embry., 21: 275-276.

Gunter, Gordon
1945. Studies on marine fishes of Texas. Pub. Inst. Mar. Sci., I: 51-53.

Hann, Harry W.
1927. The history of the germ cells of *Cottus bairdii* Girard. J. Morph., 43: 427-497.

Heyes, Florence
1931. The problem of the origin of the germ cells. Quart. Rev. Biol., 6: 1-45.

Hoffman, C. K.
1886. Zur Entwicklungsgeschichte der Urogenital Organe bei den Anamnia. Zeitschr. fur Wissenschaftliche Zoologie, 44: 570-643.

Hotta, Hideyuki
1955. On the mature Mugilid fish from Kabashima, Nagasaki Pref., Japan, with additional notes on the convolutions of Mugilidae. Jap. Journ. Ichthyol., 6: 162-169.

Jacot, Arthur Paul
1920. Age, growth, and scale characters of the mullets, *Mugil cephalus* and *M. curema*. Trans. Amer. Micr. Soc., 39: 199-299.

James, Marian F.
1946a. Histology of gonadal changes in the Bluegill, *Lepomis macrochirus* Rafinesque, and the Largemouth Bass, *Huro salmoides* (Lacépède). J. Morph., 79: 63-91.
1946b. Hermaphroditism in the Largemouth Bass. J. Morph., 79: 93-95.

Johnson, Malcolm C.
1954. Preliminary experiments on fish culture in brackish-water ponds. Prog. Fish-Cult., 16(3): 131-133.

Johnston, Perry Max
1951. The embryonic history of the germ cells of the largemouth bass, *Micropterus salmoides salmoides* (Lacépède). J. Morph., 88: 471-542.

Jones, J. W.
1940. Histological changes in the testes in the sexual cycle of male salmon parr (*Salmo salar* L., juv.). Proc. Roy. Soc., 128: 498-509.

Jordan, H. E.
1917. Embryonic history of the Loggerhead Turtle (*Caretta caretta*). Papers from the Dept. of Marine Biol. of Carnegie Inst. of Wash., Vol. XI.

Kesteven, G. L.
1942. Studies in the biology of Australian mullet. 1—An account of the fishery and preliminary statement of the biology of *Mugil dobula* Gunther. Coun. for Sci. Industr. Res. Austral. Bull., No. 157.
1953. Further results of tagging sea mullet, *Mugil cephalus* Linnaeus, on the Eastern Australian Coast. Aust. J. Mar. Freshw. Res., 4: 251-306.

Kilby, John D.
1949. A preliminary report on young striped mullet (*Mugil cephalus* Linnaeus) in two Gulf Coastal areas of Florida. Quart. Journ. Florida Acad. Sci., 2, #1: 7-23.

King, Helen Dean
1910. Some anomalies in the genital organs of *Bufo lentiginosus* and their probable significance. J. Morph., 10: 159-175.

KINOSHITA, TOSHIYUKI
1933. A new case of hermaphroditism in *Carassius auratus* (L.). Journ. Sci. Hiroshima Univer., Series B, Div. 1, Vol. 2, Art. 12.

KINOSHITA, YOSHIHARU
1936. On the conversion of sex in *Sparus longispinus* (Temminck & Schlegel). (Teleostei). Journ. Sci. Hiroshima Univer., Series B., Div. 1, Vol. 4, Art. 5.

KRAJIAN, ARAM
1940. Histological Technic. C. V. Mosby Co., St. Louis, Missouri.

LAVENDA, NATHAN
1949. Sexual differences and normal protogynous hermaphroditism in the Atlantic Sea Bass, *Centropristes striatus*. Copeia, 1949, #3: 185-194.

LILLIE, R. D.
1954. Histopathologic Technic and Practical Histochemistry. The Blakiston Co., New York and Toronto.

LIU, C. K.
1944. Rudimentary hermaphroditism in the Symbranchoid Eel, *Monopterus javanensis*. Sinensia, 15: 1-8.

LONGLEY, WILLIAM J., & SAMUEL F. HILDEBRAND
1940. New genera and species of fishes from Tortugas, Florida. Papers from Tortugas Laboratory, 32: 221-285.

LOFTS, B., & A. J. MARSHALL
1957. Cyclical changes in the distribution of testes lipids of a Teleost fish, *Esox lucius*. Quart. J. Micr. Sci., 98: 70-88.

MCKAY, DONALD, ARTHUR T. HERTZIG,
ELEANOR C. ADAMS & SARA DANZIGER
1953. Histochemical observations on the germ cells of human embryos. Anat. Rec., 117: 201-219.

MARSHALL, A. J., & B. LOFTS
1956. The Leydig cell homologue in certain teleost fishes. Nature, 177: 704-705.

MATHEWS, S. A.
1938. The seasonal cycle in the gonads of Fundulus. Biol. Bull., 75: 66-74.

MOORE, G. A.
1937. The germ cells of the Trout (*Salmo irideus* Gibbons). Trans. Amer. Micr. Soc., 56: 105-112.

NAIR, G. SIVANKUTTY
1957. Notes on the early development of *Mugil cephalus* Linnaeus. Bull. Centr. Res. Inst. Univ. of Travancore, Series C, Vol. V, #1: 77-84.

NELSON, OLIN E.
1953. Comparative Embryology of the Vertebrates. The Blakiston Co., New York and Toronto.

NEWMAN, H. H.
1908. A significant case of hermaphroditism in fish. Bio. Bull., 15: 207-214.

NIEUWKOOP, P. D.
1949. The present state of the problem of the "Keimbahn" in the vertebrates. Experientia, 5: 308-312.

ODUM, EUGENE
1936. Notes on the history of the germ cells in the Toadfish (*Opsanus tau*). Journ. Elisha Mitchell Found., Scientific Monthly, 52: 235-246.

OKA, TOHRU B.
1931. On the accidental hermaphroditism in *Oryzias latipes*. Journ. Fac. Sci., Imperial Univ. Tokyo, Vol. II, Sect. IV, pt. 3: 219-225.

OKELBERG, PETER
1921. The early history of the germ cells in the Brook Lamprey, *Entosphenus wilderi* (Gage), up to and including the period of sex differentiation. J. Morph., 35: 1-151.

VAN OORDT, G. J.
1925. The relation between the development of the secondary sex characters and the structure of the testes in the Teleost, *Xiphophorus helleri* Haeckel. Brit. Journ. Exp. Biol., 3: 43-61.
1929. Zur mikroskopischen Anatomie der Ovariotestes von Serranus und Sargus (Teleostei). Zeitschrift. f. mikr. anat. Forsch., 19: 1-17.

PICKFORD, GRACE E., & JAMES W. ATZ
1957. The Physiology of the Pituitary Gland of Fishes. New York Zoological Soc., New York.

RISLEY, PAUL L.
1933. Contributions on the development of the reproductive system in *Sternotherus odoratus* (Latreille). Zeitschr. f. Zellforsch. u. mikr. Anat., 18: 459-543.

ROBERTSON, J. G.
1953. Sex differentiation in the Pacific Salmon, *Oncorhynchus keta* (Walbaum). Canadian J. Zool., 31: 73-79.

ROUGHLEY, T. C.
1951. Fish and Fisheries of Australia. Government Printer. Sydney, Australia, 131-137.

SAMUEL, MARY
1943. Studies on the corpus luteum in *Rhinobatus granulatus* Cuv. Proc. Indian Acad. Sci., B 18: 133-157.

Sarojini, K. K.
1951. The fishery and biology of the Indian Grey Mullets. A Review. Journ. Zool. Soc. India, 3: 159-179.

Sanzo, Luigi
1936. Contributi alla conoscenza dello svillupo embrionario a post-embrionario nei Mugilidi: I—Uovo e larve di *Mugil cephalus* Cuv. Ottenute per fecondazione artificiale. R. Comit. Telassogr. Ital., 230.

Sink, E. W.
1912. The origin of the germ cells in the Toadfish, (*Opsanus tau*). Mich. Acad. Sci., Rept. #14: 212-217.

Smith, J. L. B.
1935. The Fishes of the Family Mugilidae in South Africa. Mus. Ann., 30 (19): 587-644.

Stromstein, F. A.
1931. The development of the gonads in the Goldfish, *Carassius auratus* (L.). Univ. Iowa Studies in Nat. Hist., 13, #7.

Tavolga, Margaret C.
1949. Differential effects of estradiol, estradiol benzoate and pregneninolone on *Platypoecilus maculatus*. Zoologica, 34: 215-237.

Thomson, J. M.
1949. The effects of a period of increased legal minimum length of Sea Mullet in West Australia. Austr. J. Mar. Freshw. Res., II (2): 199-220.
1951. Growth and habits of the Sea Mullet, *Mugil dobula* Gunther, in Western Australia. Austr. J. Mar. Freshw. Res., II (2): 193-225.
1953. Status of the fishery for Sea Mullet (*Mugil cephalus* Linnaeus) in Eastern Australia. Austr. J. Mar. Freshw. Res., 4: 41-81.

Turner, C.
1919. The seasonal cycle in the spermary of the Perch. J. Morph., 32: 681-705.
1928. A case of hermaphroditism in the Perch. Anat. Rec., 37: 186.

Vaupel, Jean
1929. Spermatogenesis of *Lebistes reticulatus*. J. Physiol., and Morph., 47: 555-587.

Weisel, George F.
1943. A histological study of the Sockeye Salmon (Oncorhynchus nerka). J. Morph., 73: 207-229.

Wheeler, J. F. G.
1924. The growth of the eggs of the Dabb (*Pleuronectes limanda*). Quart. J. Micr. Sci., 68: 641-659.

Witschi, Emil
1921. Development of gonads and transformation of sex in Frog. Amer. Nat., 55: 529-538.
1948. Migration of the germ cells of human embryos from yolk sac to the primitive gonadal fold. Contrib. Embryology, Carnegie Inst. Washington, XXXII, #210: 67-80.

Wolf, Louis E.
1931. The history of the germ cells in the viviparous Teleost, *Platypoecilus maculatus*. J. Morph., 52: 115-153.

Zwei, M.
1950. Typical sex-reversal in Teleosts. Proc. Zool. Soc., London, 119: 917-920.

EXPLANATION OF THE PLATES

All figures are photomicrographs of preparations from Bouin-fixed material. Unless otherwise stated, the sections were stained with Galigher's Alum Hematoxylin and Triosin.

Plate I

Fig. 1 & 1a. Transverse section through the posterior abdominal region of a 23 mm. mullet fry. The arrow in Fig. 1 indicates the general position of the developing gonad between the abdominal cavity and the peritoneum. In Fig. 1a, the two anlagen are shown suspended from the black pigmented peritoneum and separated by the dorsal mesentery. The right anlage contains a small area of syncytial cord tissue. 24× and 530×.

Fig. 2. A 5-micra section through a genital strand of a 20 mm. fry. The large germ cell in the primordium measured approximately 12 micra on its long axis. 1300×.

Fig. 3. A 5-micra section (transverse) of one gonad from a 50 mm. fry. Note that the germ cells are limited to the lateral side and ventral end (left and bottom in the figure); also that the epithelial border is conspicuous on the lateral margin and lacking on the medial side. 1140×.

Fig. 4. A section through the dorsal mesentery from the posterior region of a 50 mm. fry. The peritoneum is at the top and the gut at the bottom of the figure. The two knob-like projections from the dorsal mesentery represent the genital strands in transverse section. 240×.

Plate II

Fig. 5. The arrangement of the germ cells around the lateral and ventral periphery (right and bottom) and the development of nests is indicated in this section from a 61 mm. specimen. The bottom of the figure shows top-shaped nests, an indication that some of the germ cells have migrated toward the center. Note the branched ducts which have penetrated to the peripheral nests. 495×.

Fig. 6. A transverse section of one gonad from a 75 mm. fish. The germ cells appear in nests along the lateral and ventral regions (left and bottom). The arrow indicates the hilus in which involution of the epithelial border is taking place. A 7-micra section. 450×.

Fig. 7. A transverse section from a 100 mm. mullet. The central or main duct has formed and appears as a narrow fissure in the figure.

Fig. 8. About one-half of a section of the gonad of a 150 mm. fish, collected in May, is shown. To the right and below center is a small, dark oocyte-like cell. 525×.

Plate III

Fig. 9. A low power view of a transverse section through the maturing testis of a 250 mm. mullet, collect in August. The tubules, containing the developing male cells, which radiate from the vas deferentia, are the light, columnar areas. Masson C. T. stain. 53×.

Fig. 10. Detail of several tubules from a testis similar to that in Fig. 9. A 7-micra section. Masson's C. T. stain. 525×.

Fig. 11. Maturation of the germ tissue in the peripheral region of a testis from a specimen collected in October. A 7-micra section. 525×.

Fig. 12. A general view of the peripheral region of a near-ripe testis collected in late October. The engorgement of the tubules with spermatids and sperm has reduced the stroma of the gland to no more than strands of tissue. A 5-micra section. 175×.

Fig. 13. A small area of a tubule of a near-ripe testis. A 5-micra section. 250×.

Plate IV

Fig. 14. An area from a spent testis of a 215 mm. fish. Note the two ova-like cells (right) and the germ cells in the circled area. A 7-micra section. 250×.

Fig. 15. A low power view of an area from a spent testis collected in February (275 mm. standard length). The crenated edge of the gland and the collapsed tubules are characteristic of the spent testis. A 7-micra section. 53×.

Fig. 16. An ova-like cell surrounded by sperm. The region shown is the vas deferens. From the same fish as Fig. 15. A 7-micra section. 495×.

PLATE V

FIG. 17. A transverse section of a developing ovary from a 200 mm. specimen collected in February. The arrangement of the germ cells in nests is characteristic of the presumptive ovary. A 7-micra section. 185×.

FIG. 18. A low power view of a more mature ovary. This specimen measured 200 mm. and was collected in February. Note that the duct that separates the body of the gland from the mesovarium, which forms the vas deferens in the male, is still present. A 7-micra section. 53×.

FIG. 19. A small area from the same ovary as Fig. 18. There are nests of maturing gonia cells and oocytes in various stages of development. A 5-micra section. 250×.

PLATE VI

FIG. 20. A low power photograph of a section through the peripheral region of an ovary collected in February. The general pattern of the lamellae is shown. The tunica in this region is still made up of two layers of collagenous connective tissue. (An area from a region near the mesorchium would have shown smooth muscle in the tunica). Note the pigmented serosa. A 7-micra section. 53×.

FIG. 21. Sections through several near-ripe oocytes from the ovary of the largest female fish examined, *i.e.*, 425 mm. This fish was collected on February 15. The dark, irregularly-shaped structures near the top of the figure are young oocytes which have been distorted by the pressure of the larger oocytes. A 7-micra section. 250×.

FIG. 22. A higher magnification view of the cortical regions of three ova from the same specimen as the previous figure. Only two structures appear to be outside the cytoplasm of the egg, a zona radiata and the follicle cells. The round clear areas in the cytoplasm are presumably the result of oil dissolution. Masson's C. T. stain; 6-micra section. 525×.

PLATE VII

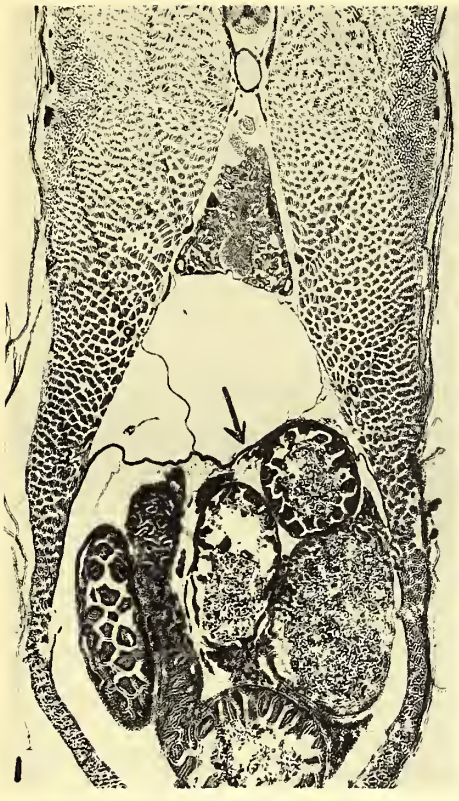
FIG. 23. A low power view of a section through the ovary of a spent fish. A 7-micra section. 53×.

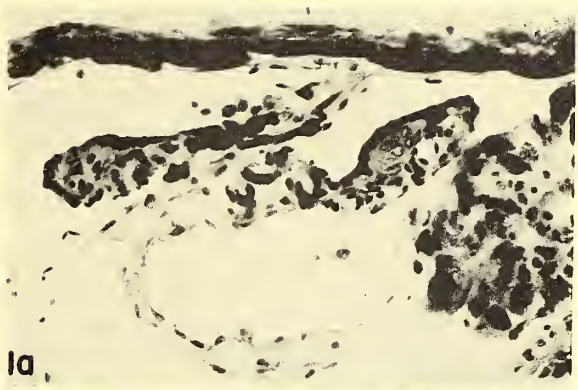
FIG. 24. A small area from a spent ovary of a fish of 275 mm. collected in February. Between the dark oocytes are two or three nests of germ cells, probably oogonia. A 7-micra section. 525×.

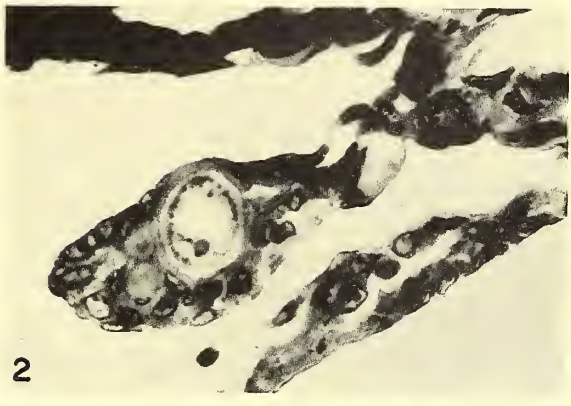
PLATE VIII

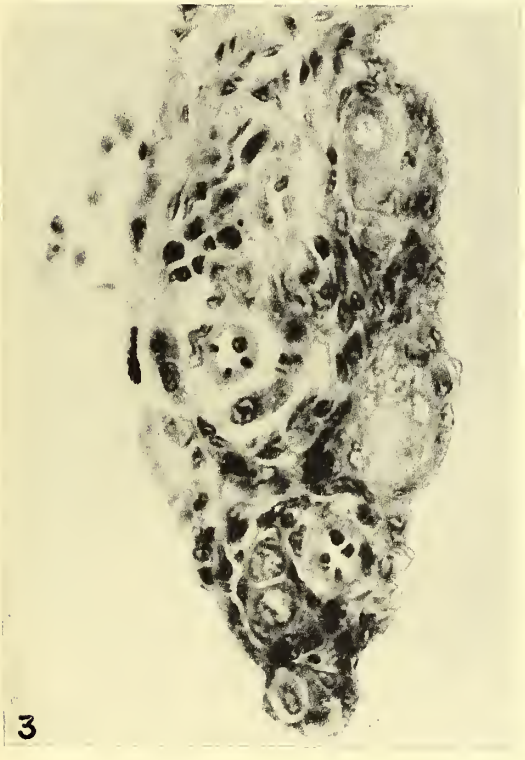
FIG. 25. A low power view of a transverse section through one gonad of an hermaphrodite specimen. It was collected on October 8. A 7-micra section. 53×.

FIG. 26. From the above specimen, showing spermatids and sperm in the testicular part of the gland. Note the dark ova or oocyte-like cells as well as normal-appearing sperm "parachutes". A 7-micra section. 600×.

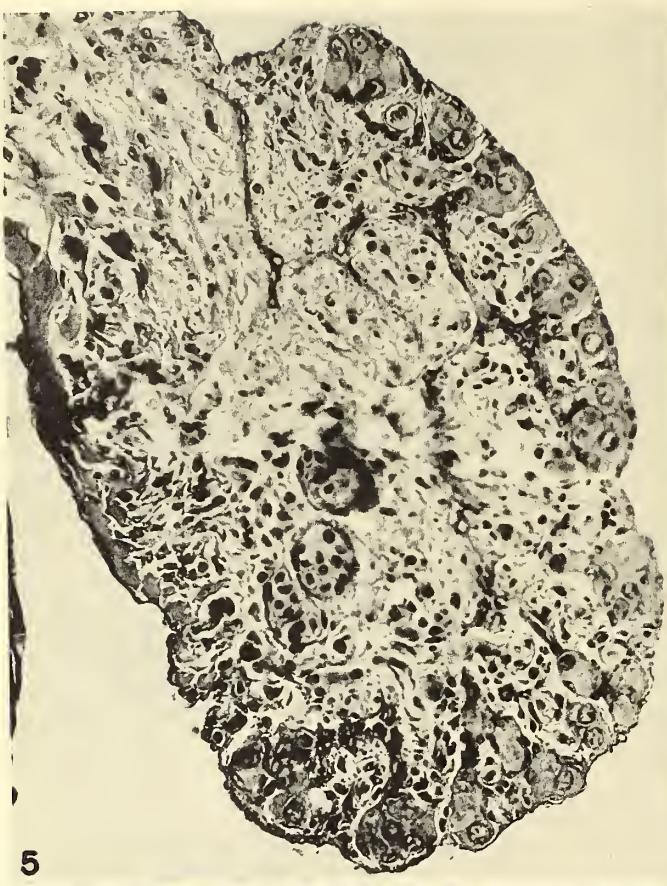




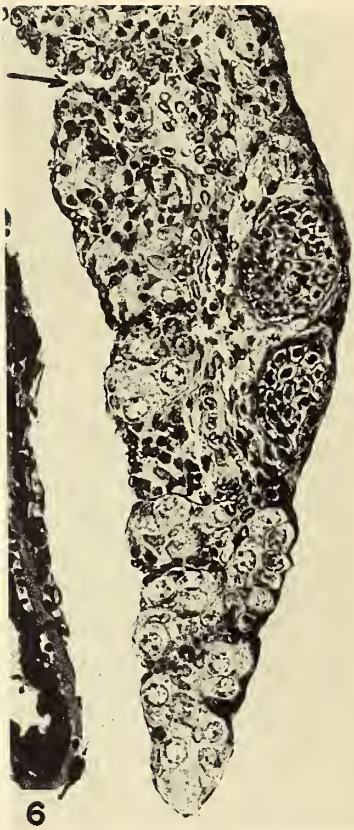




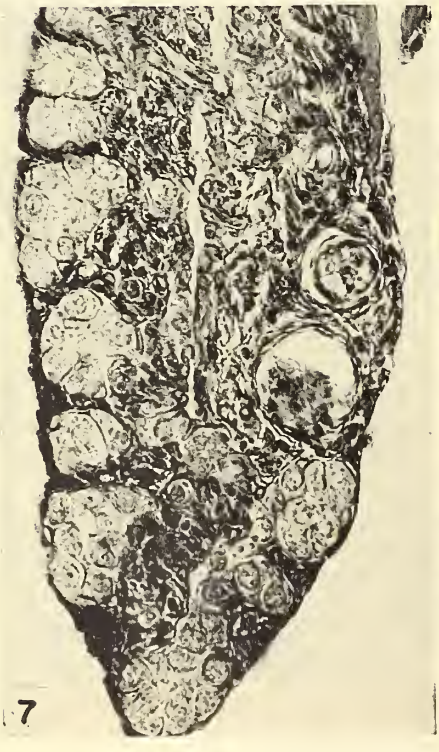
A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS



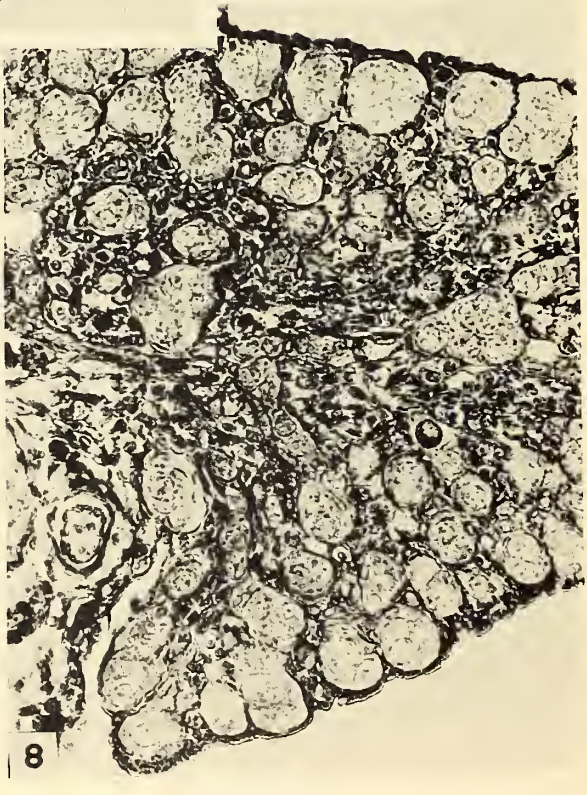
5



6

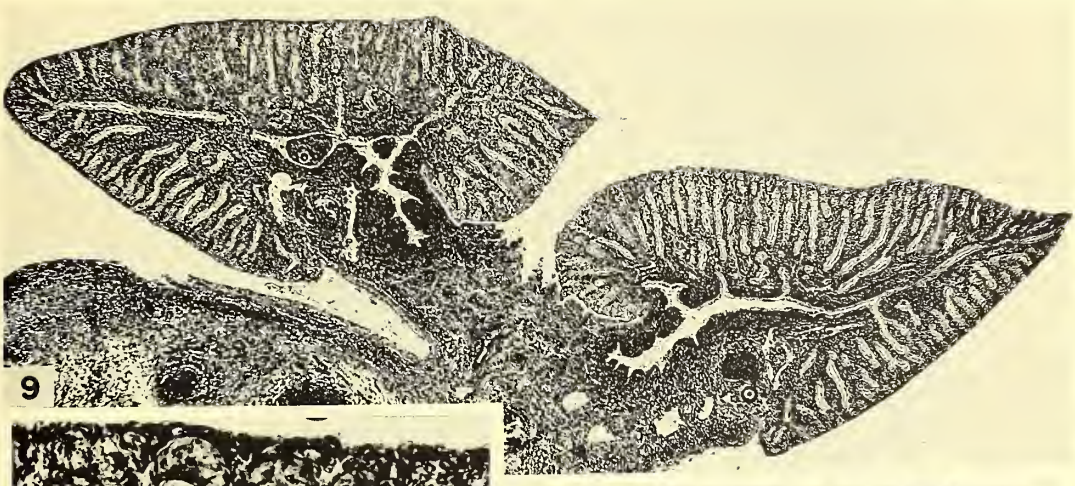


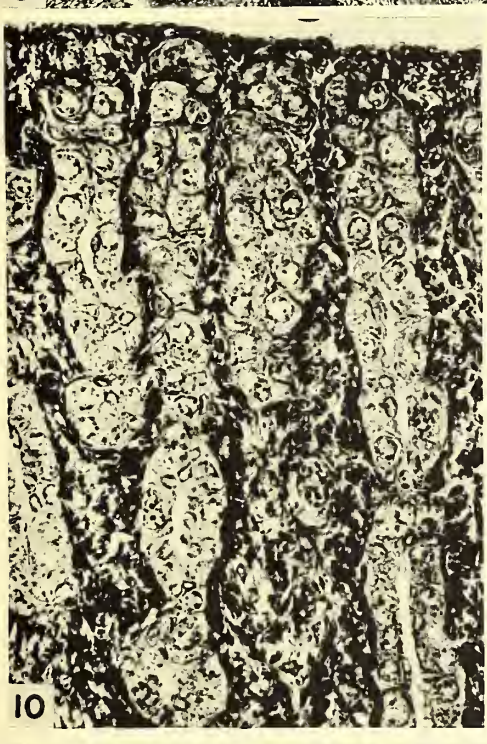
7

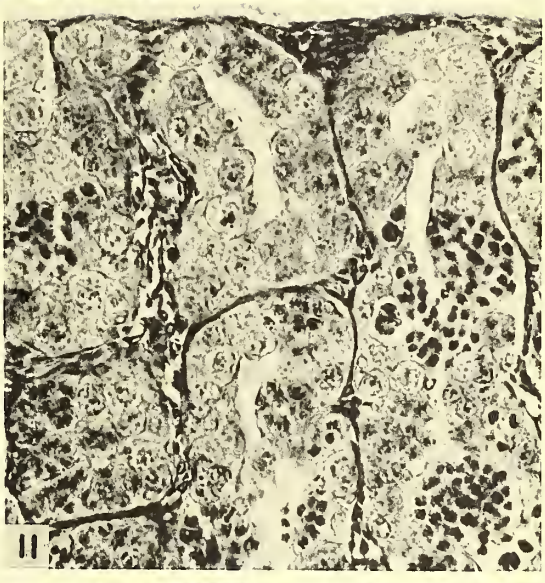


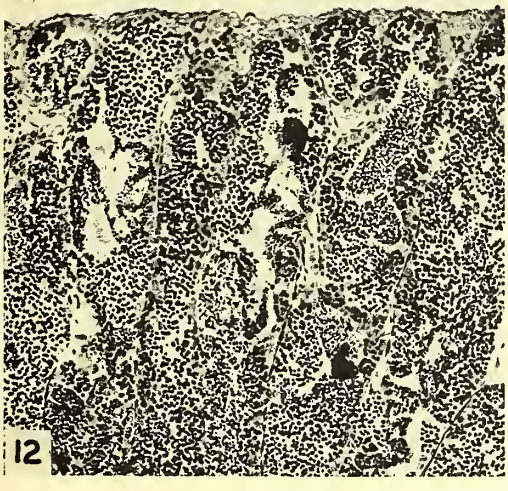
8

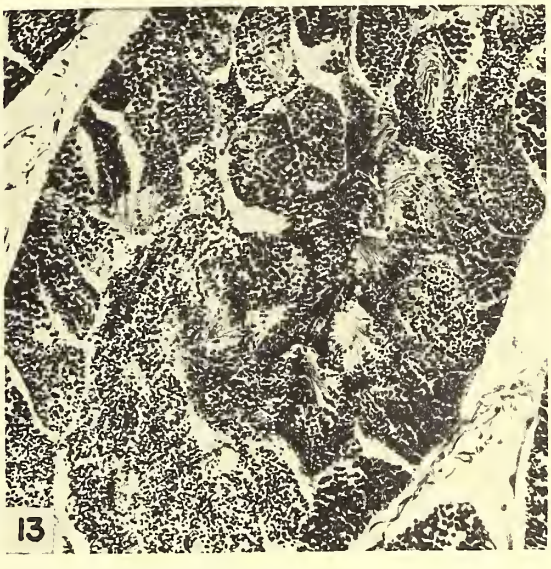
A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS



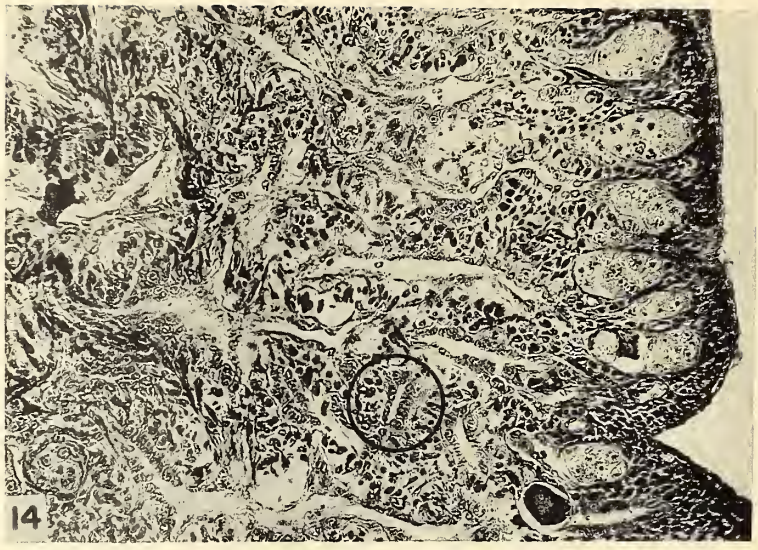


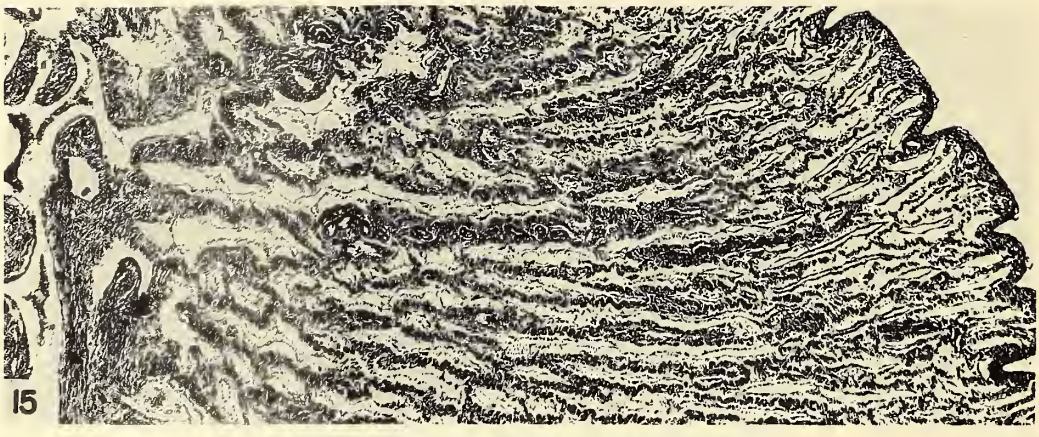


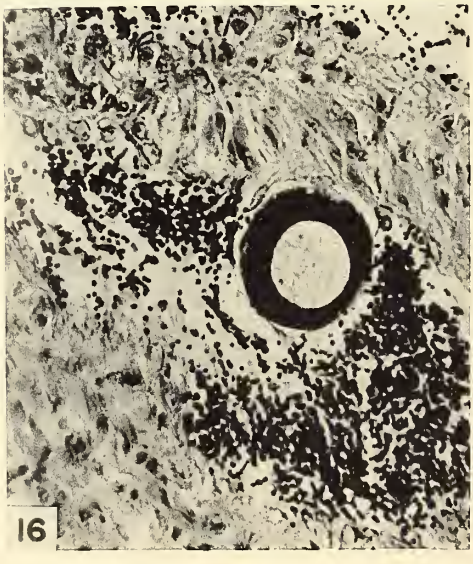




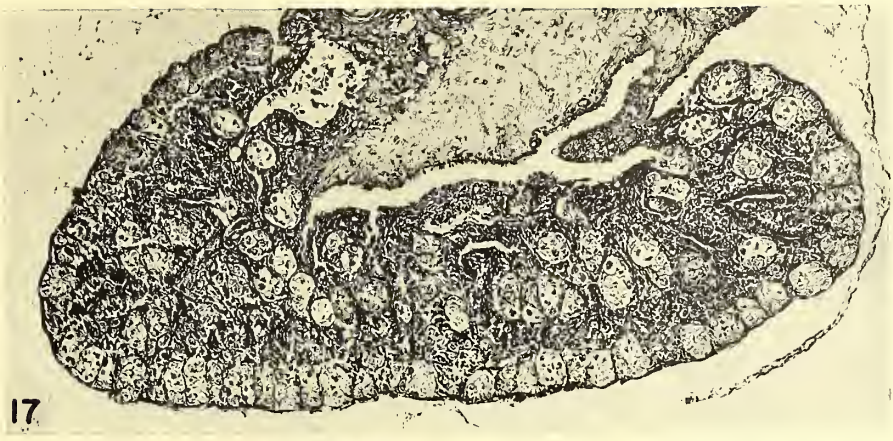
A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN
REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS



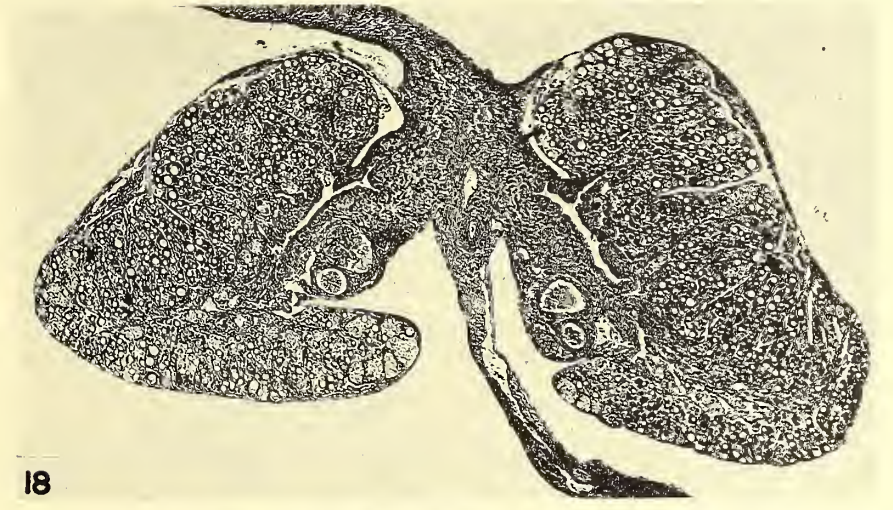




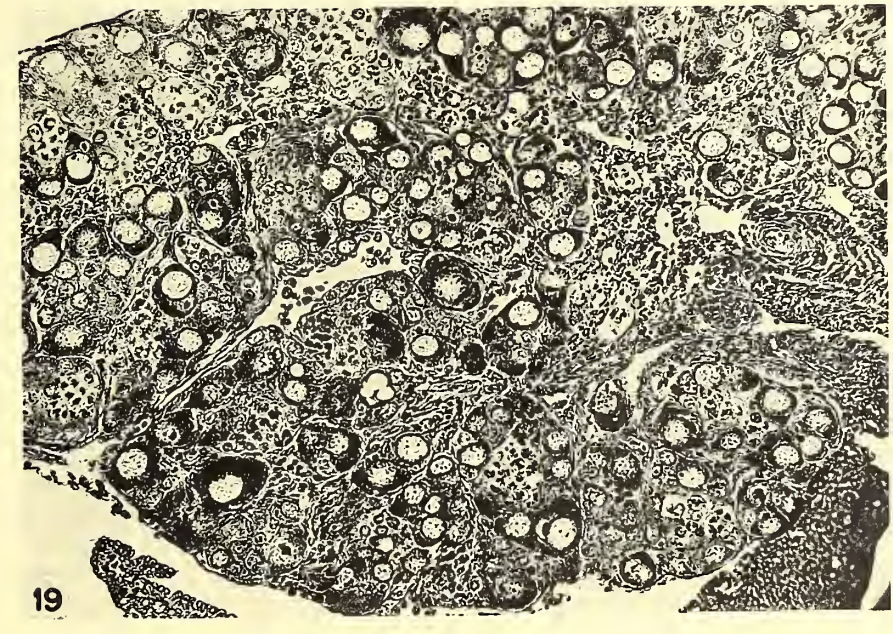
A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS



17

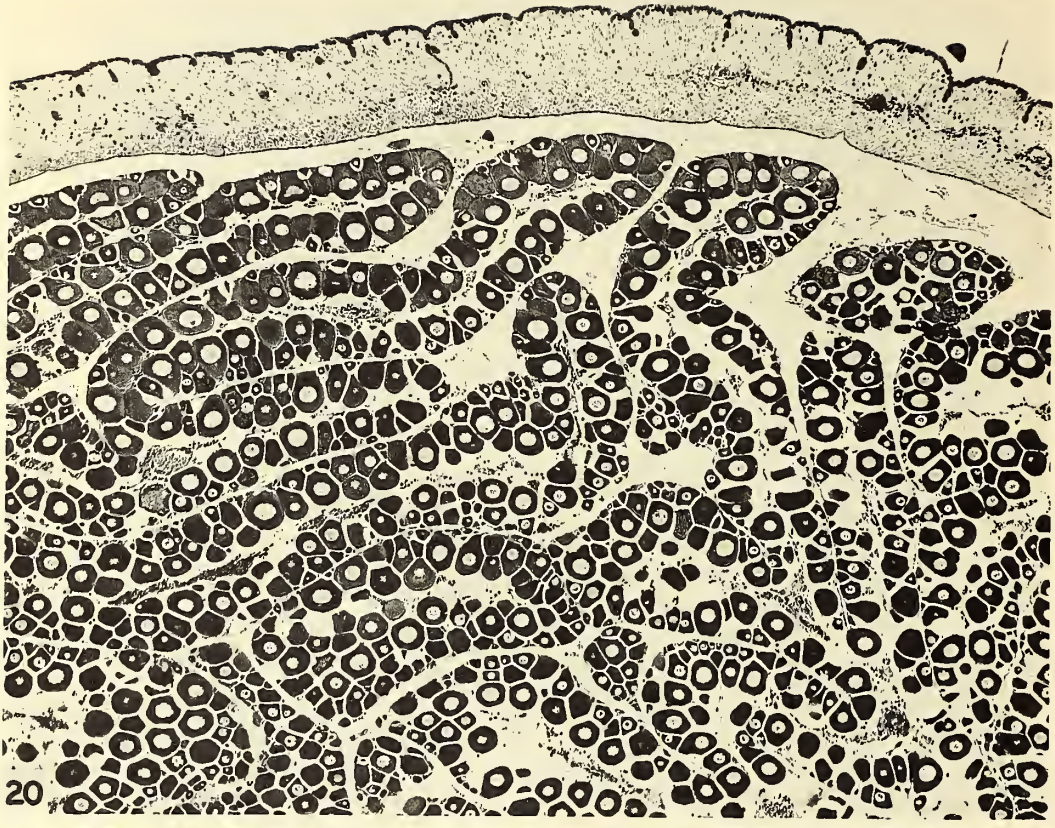


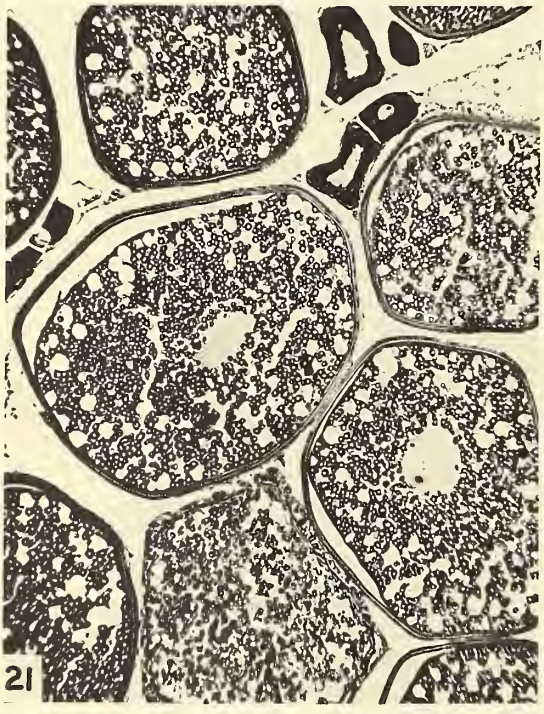
18

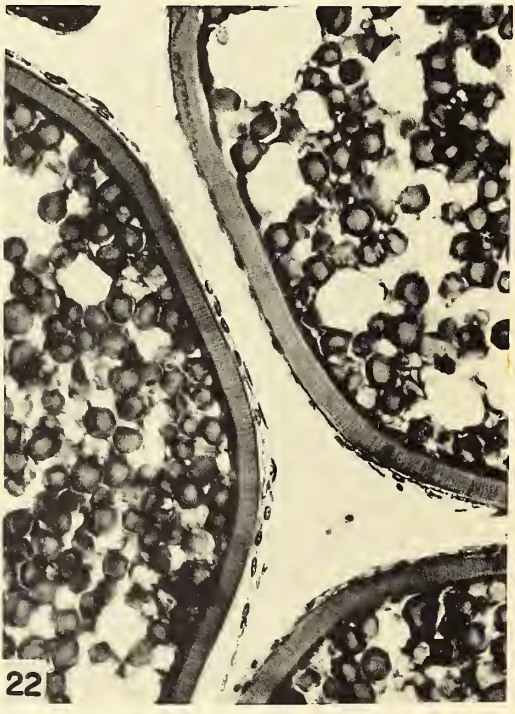


19

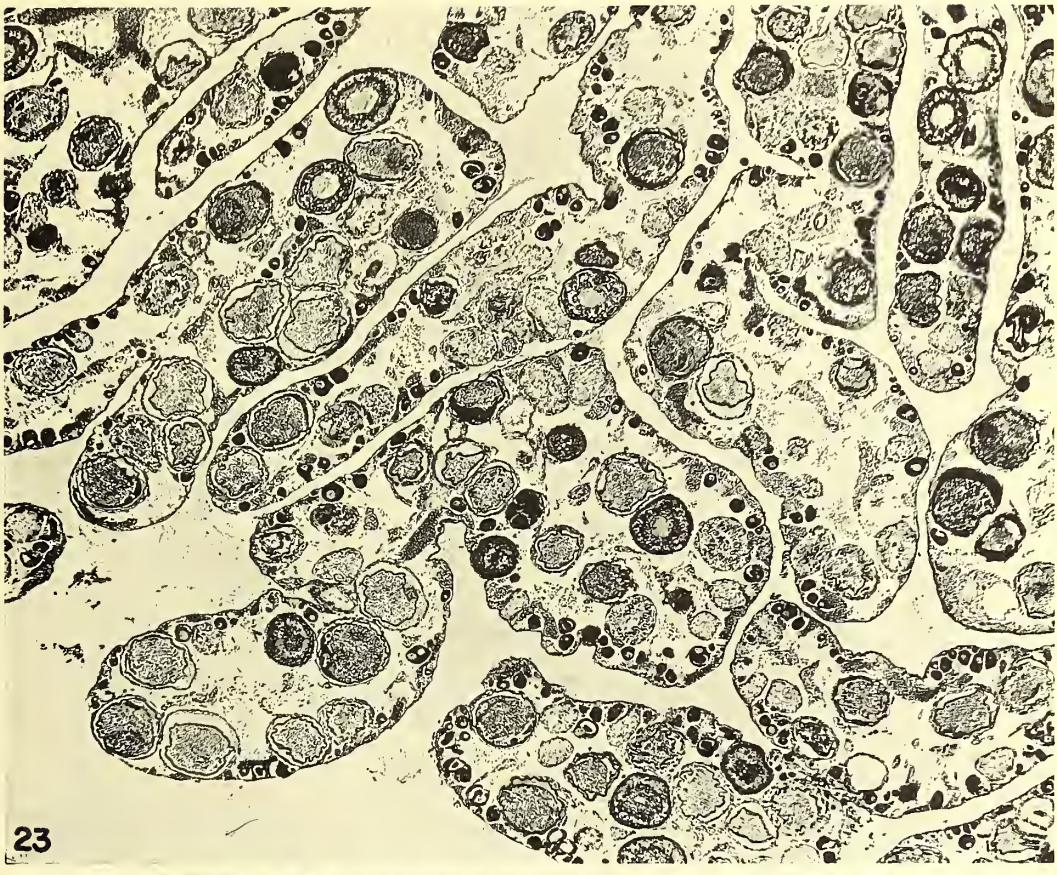
A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN
REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS

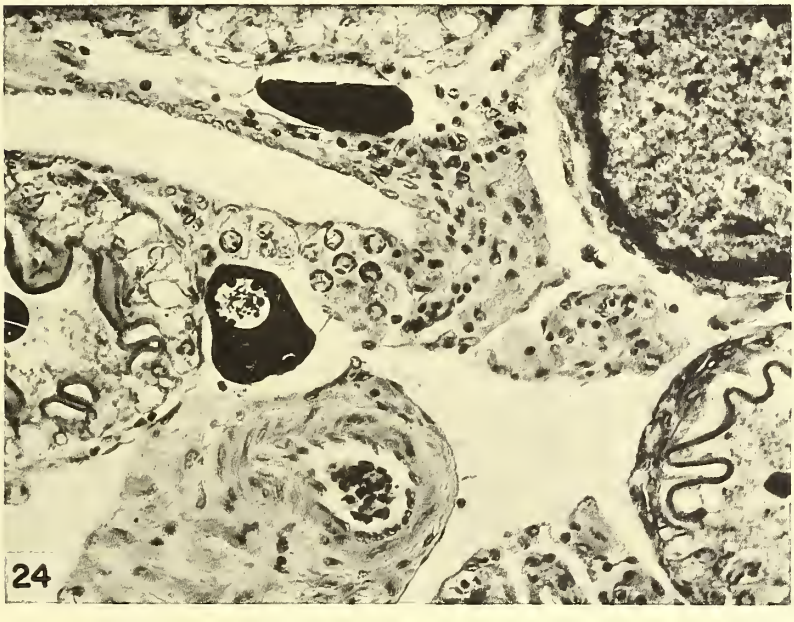




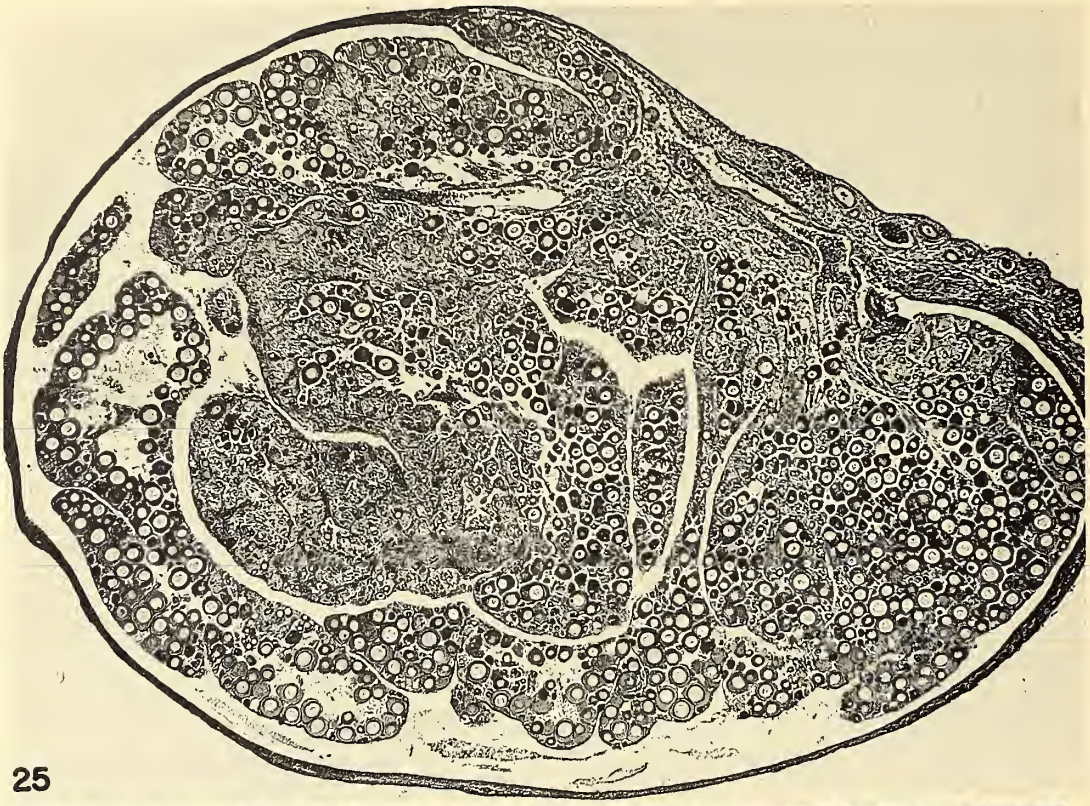


A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN
REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS

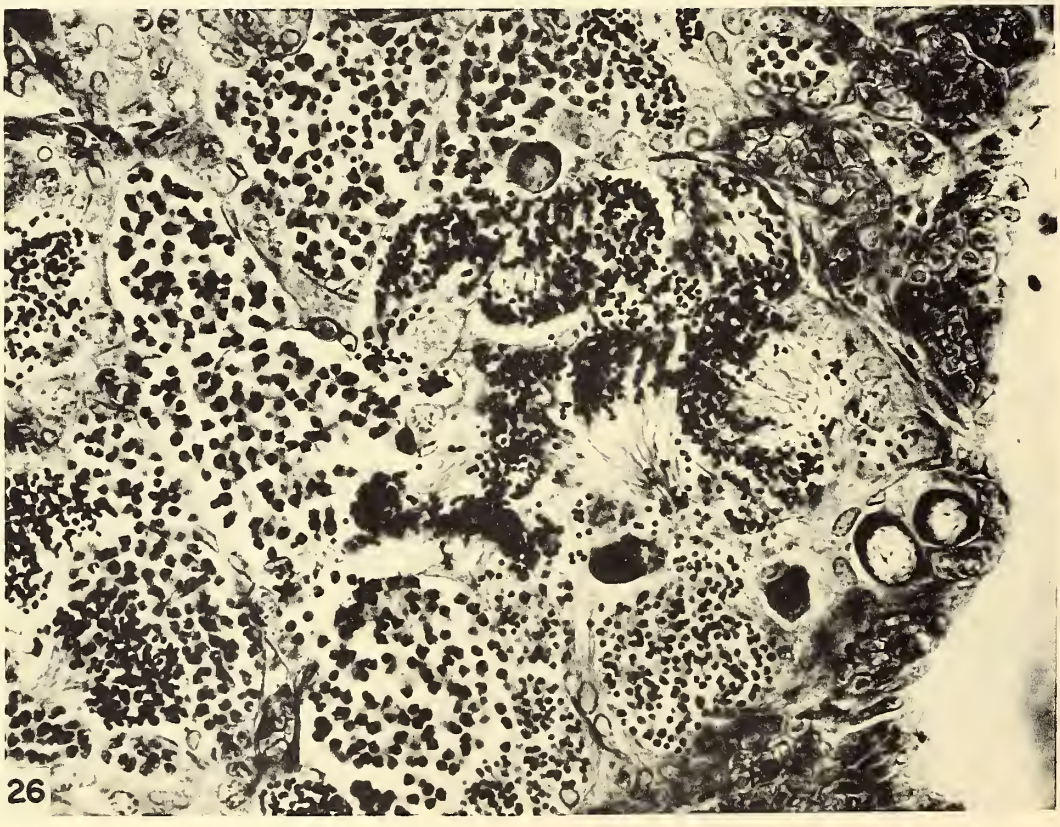




A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN
REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS



25



26

A STUDY OF THE STRUCTURE AND DEVELOPMENT OF CERTAIN
REPRODUCTIVE TISSUES OF MUGIL CEPHALUS LINNAEUS

4

The Production of Underwater Sound by *Opsanus* sp., a New Toadfish from Bimini, Bahamas[1,2,3]

MARIE POLAND FISH
&
WILLIAM H. MOWBRAY
Narragansett Marine Laboratory,
University of Rhode Island

(Plates I-III; Text-figs. 1-5)

INTRODUCTION

DURING investigations into the occurrence, character and significance of underwater sounds produced by subtropical marine species, two toadfishes were auditioned at the Lerner Marine Laboratory, Bimini, Bahamas, in December, 1952. These specimens were temporarily labelled *Opsanus tau*[4], but the fact that the recorded sounds differed from those of the many *O. tau* already studied in continental waters was noted in our report. Similar soundmaking by Bimini toadfishes was recorded in December, 1956, and April, 1957. Acordingly these alternatives were presented: (1) sound production within one species varies geographically far more than previously supposed; or more probably, (2) the experimental specimens were not *O. tau.*

That Bimini toadfishes are not *O. tau* but a previously undescribed species, as subsequently established by Walters & Robins (MS), suggests that in some cases quality and pattern of underwater sounds may constitute a diagnostic specific character. Such differentiation can be of particular value toward identification of field contacts.

EXPERIMENTAL PROCEDURE

All specimens were collected at low tide from the shallow flats of Tagus Key, within the Bimini lagoon. Immediately after capture they were transferred to glass aquaria for close observation and frequent monitoring, and during actual recording to a wooden tank insulated by a thick lining of Hairflex, rubberized horsehair.

The 1952 monitoring system consisted of a NOL Type 1-A, Model Q4, quartz crystal hydrophone with an amplifier modified from JO Underwater Sound Equipment. Recordings were made on a Magnecord PT63 at a tape speed of 15 inches per second. During 1956-57 a more compact AN/SSQ-2 barium titanate hydrophone, modified for this purpose by the authors, was used. Recordings at fixed locations were made on a Magnecord F35-B, and a Magnemite

[1]Walters & Robins (MS) have given this species the manuscript name *Opsanus phobetron.*

[2]Contribution No. 21 from the Narragansett Marine Laboratory of the University of Rhode Island, and a Contribution from the Lerner Marine Laboratory of the American Museum of Natural History. This paper is based on research conducted under Contract Nonr-396(02) between the Office of Naval Research and the Narragansett Marine Laboratory. In connection with studies on sound production in western Atlantic waters, 125 species of fishes were auditioned at Bimini by the authors.

[3]Sincere appreciation is extended to the Lerner Marine Laboratory for generous cooperation and excellent facilities provided during two field programs, and to Dr. Charles J. Fish, Director of the Narragansett Marine Laboratory, for valuable advice and assistance throughout all phases of the project. The authors gratefully acknowledge the services of Prof. Robert A. DeWolf as consultant in anatomical problems.

[4]Rough identification was based on reticulated color pattern and distribution, which favored *O. tau,* the only toadfish reported along the Atlantic coast from Maine to Cuba, rather than *O. beta,* a Gulf of Mexico species known to stray rarely as far north as Biscayne Bay.

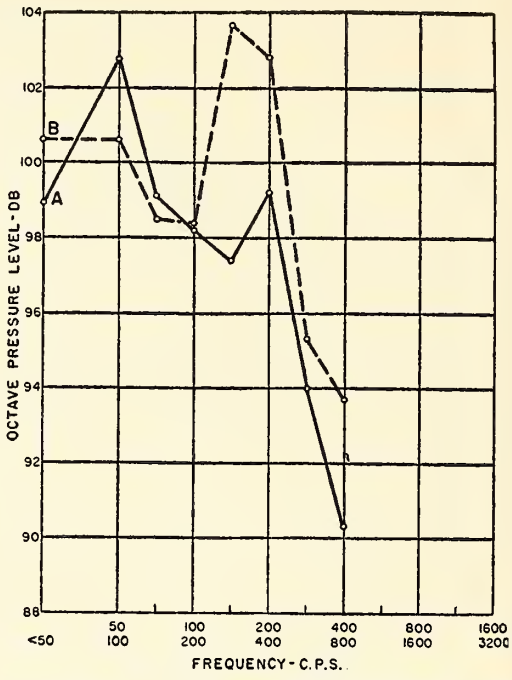
610-VU battery-spring operated recorder was used for field listening and recording.

Frequency analysis of the recorded sounds was accomplished by three methods. The pressure amplitude distribution was determined by a Western Electric RA-363 filter set having 12 overlapping octave ranges. A General Radio 760-B sound analyzer was used to separate the major individual frequency components. Photographs of the sounds displayed on a Dumont 304-A oscillograph were used with a Dumont 296 oscillograph camera to allow detailed examination and comparison, and to provide accurate time and frequency measurements.

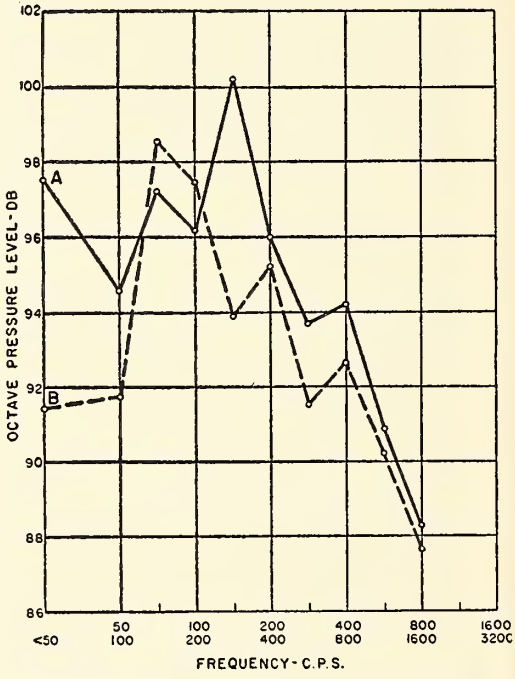
Recorded Sounds

Type I Sound.—An immature female (specimen #1) measuring 165 mm. in total and 140 mm. in standard length, which had been held under observation for three weeks[5], was used for experiment on December 1, 1952. Routine stimulations involving changed environment, mild aggravation and extreme duress gave negative results; no biological sound could be stimulated thus. However, when electrically stimulated by 60 cycle a-c shock, a low vibrant grunt was consistently produced, coincident with each instantaneous shock. These apparently involuntary sounds exhibited a fundamental frequency of 60 cycles with strong second and fourth harmonics; all harmonics to the tenth were measurable.[6]

As shown by Text-figs. 1 & 2, this type of Bimini toadfish growl is very similar in frequency pattern to the characteristic coarse growl of *O. tau.* However it is a more sustained and less raucous sound. Over-all pressures of 104.7 to 111.05 db above 0.0002 dyne/cm^2 were registered at a distance of two feet from the hydrophone; under the same electric stimulation *O. tau* sounds regularly measured under 106 db and only rarely reached 108 db. These slight differences between the two species are not significant since intensity, influenced by size of individual and degree of stimulation, may be expected to vary considerably within a single species.



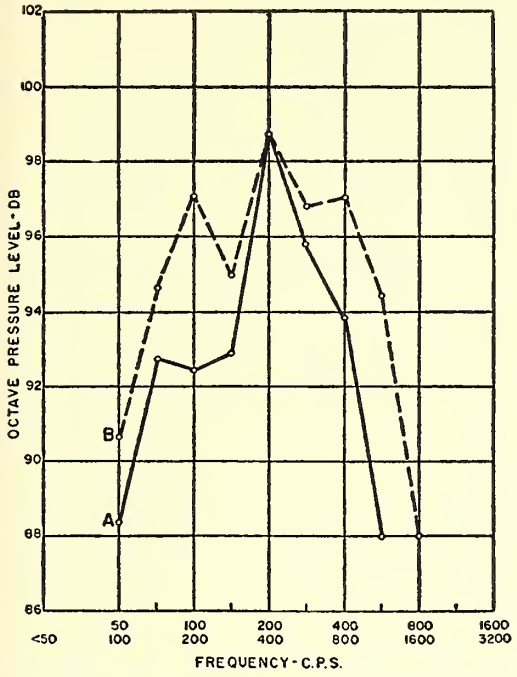
Text-fig. 1. Type I sounds of specimen #1, an immature female recorded at Bimini in 1952. Curve **A**: average of 4 sounds, over-all pressure 104.7 db. Curve **B**: average of 2 sounds, 104.9 db.



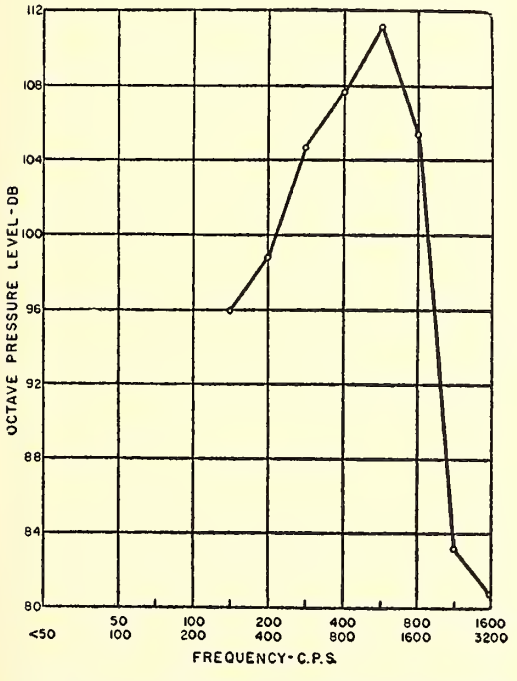
Text-fig. 2. Composite of sounds produced by 7 specimens of *O. tau* recorded in Rhode Island in 1951. Curve **A**: average of 10 sounds from 3 specimens. Curve **B**: average of 15 sounds from 5 specimens.

[5]Because of the tendency of most fishes to remain silent when confronted with strange situations, especially in the presence of vessels and underwater gear, an attempt is made to acclimate specimens to the experimental area and equipment over as long a period as possible.

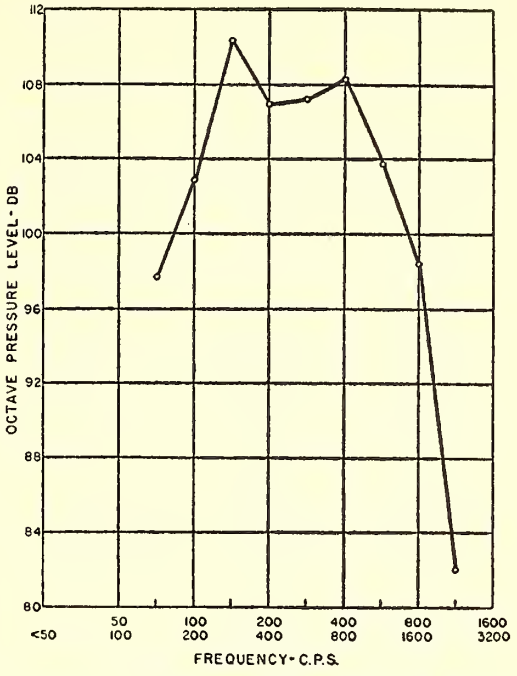
[6]In the case of *O. tau* (Fish, 1954), electrically induced grunts differed from the spontaneous grunts only in a widening of frequency range; the fundamental frequency was predominantly the same. Experiments using electrical stimulation with d-c and a-c of various frequencies indicated that the natural fundamental frequency of the sounds was not a result of the 60 cycle stimulation.



TEXT-FIG. 3. Type II sounds of specimen #2, recorded at Bimini in 1952. Curve **A**: average of 2 sounds, over-all pressure 100.9 db. Curve **B**: average of 2 sounds, 102.3 db.



TEXT-FIG. 4. Average of 10 sounds from specimen #3, a mature male recorded at Bimini in 1956. Over-all pressure 111.7 db.



TEXT-FIG. 5. Sounds from specimen #5, an immature male recorded at Bimini in 1956. Average of 15 sounds, over-all pressure 110.95 db.

Type II Sound.—Specimen #2, measuring 115 mm. in total and 100 mm. in standard length, was subjected to the same series of stimulations on December 17, 1952. When handled, prodded gently or aggravated to attack, a more protracted and rather musical burst was emitted. The same sound was produced by electric stimulation but, unlike the Type I sound which was coincident with shock, occurred only after release from shock. The fish reacted to small instantaneous shock by a slight muscular shudder and extension of opercula and fins, followed, after release from shock, by the rumbling burst. (Text-fig. 3).

Of the three 1956-57 toadfishes, only two could be induced to sound production. On December 21, 1956, long bursts were emitted immediately and over a long period when a male with well developed gonads (specimen #3) was caught in the meshes of a net; its total length was 121 mm. and standard length 105 mm. On April 15, 1957, similar bursts were recorded from an immature male (specimen #5) measuring 110 mm. in total and 89 mm. in standard length. However, on January 3, 1957, a female with partially spent ovaries (specimen #4), measuring 89 mm. in total and 80 mm. in standard length, could not be stimulated to sound-making, even by extreme duress. Whether her silence was due to sex, breeding condition or

merely individual reticence cannot be determined without data from more specimens.

The sounds produced by specimens #3 and #5 differ considerably from any previous toadfish recordings. Those of the mature male (#3) range between 634 and 800 cps, and thus are much higher in frequency than the normal grunts of this species, the grunts and boat-whistles of *O. tau* (Fish, 1954) and of *O. beta* (Tavolga, 1958). Analysis of these sounds (Text-fig. 4) shows very little harmonic content, which is confirmed by the comparatively good wave form on the oscillographic record (Plate I, Fig. 1). A small second harmonic is measurable.

Closer observation indicates that many sounds heard as a single pulse actually consist of two distinct pulses separated by a short interval (Plate I, Fig. 2). While these pairs have the same general characteristics as the single sounds, the repetition frequency and duration are more variable within each group and among different groups. The sounds may occur as isolated single or paired pulses, but usually occur in rapid bursts of two or more groups containing random combinations of single and paired pulses. The shorter bursts consist of less than 10 groups produced at rates ranging from four to nine groups per second, averaging about five. In longer bursts, the groups are produced at a slower rate; the longest burst measured 38 seconds duration and contained 82 groups of both single and paired pulses.

To the unaided ear the sounds of the smaller, immature male #5 appear quite similar to those of the mature male #3, but analysis shows a much lower fundamental frequency with many harmonics to about 1100 cps (Text-fig. 5). The probable fundamental ranges between 105 and 145 cycles, comparable to the Type II sound of the 1952 specimens, and is weak in comparison to large second and fourth harmonics. The oscillographic records show these complex wave forms with apparent repetition frequencies between 217 and 286 cps, *i.e.,* second harmonics (Plate I, Fig. 3).

This specimen produced the same double-pulse sounds as #3, with even greater variations. As shown in Plate I, Fig. 4, and Plate II, Figs. 5 & 6, the sound duration, principal frequency and wave form change within any given group, and no two groups are identical. Detailed analysis of a single-pulse burst indicates a number of harmonic components differing in frequency by only a small percentage of the fundamental frequency, similar to the difference observed in the double sounds.

Because the sound-stimulating intrinsic muscle masses meet posteriorly but do not fuse (see p. 75), it is possible that contraction of the two sides of the air bladder are not always coincident, and could, therefore, result in a double sound. Moulton (1956) suggested a slightly asynchronous contraction of the two drumming muscles of the sea robin (*Prionotus* spp.) as the probable cause of a paired arrangement of pulses in its breeding season calls.

Although externally the bilobed air bladders of sea robin and toadfish are much alike, their internal structure differs. In the sea robin the two lobes are more narrowly connected, forming dissimilar compartments, whereas those of the toadfish open widely and function as a single chamber (Plate III, Fig. 9). Under these conditions, less variation would be expected in toadfish double sounds, and the slight difference in frequency content betwen the two parts of such sounds might be explained accordingly. On the other hand, Moulton recorded a difference of 1,000 cycles in intensity peaks of sea robin double sounds, "probably related to a differential resonance of the two air bladder lobes which generally differ somewhat in size." This is a much greater variation than has hitherto been observed in any sounds of air bladder origin, even among variously sized individuals within the same species. For instance, principal frequencies exhibited by large and small specimens of the eel, thread herring, striped bass, gray squeteague, tautog, spadefish and New England toadfish usually vary by not more than 100 cycles (Fish, 1954). Therefore, the present authors consider that some other factor must have contributed to the reported 1,000 cycle difference in sea robin double sounds.

Although considerable underwater listening was carried on during December, 1956, and January, 1957, in areas where Bimini toadfish breeding had been observed in previous years and might again be expected, no sounds resembling the boat-whistle blast produced in Narragansett Bay by *O. tau* (Fish, 1954) during its early spawning season were recorded (Plate II, Fig. 7).

Tavolga (1958) recorded similar boat-whistle sounds on the Florida Gulf coast in late August, 1957, presumably produced by *O. beta,* a toadfish which breeds there during the winter months. Since no blasts of this type were detected during June, July and the first 20 days of August, he suggests that such soundmaking may precede the actual breeding period. Fish (1954) reported the *O. tau* boat-whistle sounds to be associated with pre-spawning activity of individuals, and to occur in the vicinity of the Rhode Island breeding grounds only during the

early part of the season; it was considered, therefore, as a possible mating call.

If the Bimini species is capable of such soundmaking, as seems probable, failure to record it could be due to scarcity of toadfish in those years or to the late start of field observations.

Method of Sound Production

The sound-producing mechanism of the Bimini toadfish is provided by a large air bladder comprising approximately two-thirds of the visceral cavity; its mid-dorsal surface is rather loosely attached to the kidneys and margins are connected by peritoneum with the dorsal body wall. Anteriorly the air bladder is bilobed, with medial surfaces contacting each other in the mid-line. Each lateral wall is completely covered by a thick muscle mass with striated fibers running at right angles to its length; these intrinsic muscles meet but do not fuse posteriorly. Dorsal and ventral bladder surfaces are composed of tough fibrous tissue (Plate III, Fig. 8). Internally the two lobes unite to form a common chamber, walled posteriorly by a thin and somewhat vascular membrane. This transverse septum, which separates approximately one-third of the bladder into a posterior chamber, is perforated by a small central aperture (Plate III, Fig. 9). Except for greater prominance of red glands in the few specimens examined to date, the Bimini toadfish air bladder resembles that of *O. tau* both externally and internally.

Extensive experiment with *O. tau* has indicated (Fish, 1954) that sound is produced when, by contraction and expansion of the intrinsic muscle fibers, the taut membranous walls and gaseous contents of the air bladder are set in vibration. Probably sound waves are strengthened when the internal transverse septum is vibrated further by gas forced through its tiny aperture. Participation of pharyngeal teeth in ordinary sound production, although possible, has not been indicated in this species. Apparent similarity in sonic apparati leads to the conclusion that methods of sound production are identical in the two species of *Opsanus*.

Conclusions

Two general types of sound have been recognized for males of the Bimini toadfish; data are incomplete for female specimens. These sounds are sufficiently different from other *Opsanus* species to permit their use in making identifications, especially under field conditions where morphological comparisons are impossible. The chief distinction evident without physical analysis, is rapid repetition of many sound pulses into a protracted burst, whereas *O. tau* and *O. beta* soundmaking features impulsive single or double pulses of sound.

On the basis of these records, it is suggested that the quality and pattern of underwater sounds may in some cases be included in taxonomic descriptions as a diagnostic specific character.

Determination of several characteristic patterns in the present *Opsanus* recordings have indicated that a single specimen may be capable of a variety of sounds, all produced by the large and heavily-muscled air bladder.

Experimental results indicate that biological underwater sound in this species is purposeful and occurs only in response to definite physical or physiological stimulation, as reported for many other fishes (Fish, 1954). The absence of the boat-whistle sounds associated with prespawning behavior of other *Opsanus* species may be due to a current scarcity of toadfish or to the late start of field observations.

Bibliography

Fish, M. P.

1948. Sonic fishes of the Pacific. Pacif. Ocean. Biol. Project, Tech. Rep. 2: 1-144. (Woods Hole Ocean. Inst.).

1953. An outline of sounds produced by fishes in Atlantic coastal waters, sound measurements and ecological notes. Ocean. Biol. Project, Spec. Rep., 1: 1-21. (Narragansett Mar. Lab.).

1954. The character and significance of sound production among fishes of the western North Atlantic. Bull. Bingham Ocean. Coll., 14 (3): 1-109.

Fish, M. P., A. S. Kelsey, Jr., & W. H. Mowbray

1952. Studies on the production of underwater sound by North Atlantic coastal fishes. J. Mar. Res., 11 (2): 180-193.

Moulton, J. M.

1956. Influencing the calling of sea robins (*Prionotus* spp.) with sound. Biol. Bull. 3 (3): 393-398.

Naval Ordnance Laboratory

1944. Investigations of biological underwater background noises in vicinity of Beaufort, N. C. (10B1). NOL Rep., 880: 1-22. (U. S. Navy Yard).

Tavolga, W. N.

1958. Underwater sounds produced by two species of toadfish, *Opsanus tau* and *Opsanus beta*. Bull, Mar. Sci. of the Gulf and Caribbean, 8 (3): 278-284.

Tower, R. W.
1908. The production of sound in the drumfishes, the sea robin and the toadfish. Ann. N. Y. Acad. Sci., 18: (5) pt. II: 149-180.

Walters, V. & C. R. Robins
MS. A new toadfish (Batrachoididae) regarded as a glacial relict in the West Indies.

Plate I

Fig. 1. Single sound of specimen #3. Duration, 41 milliseconds; 26 cycles present, damped in groups of 3; average repetition rate, 634 cps.

Fig. 2. Double sound of specimen #3. Total duration, 43 milliseconds. First pulse: 14 milliseconds, 10 cycles present, average repetition rate, 714 cps. Second pulse: 18 milliseconds, 15 cycles present, average repetition rate, 789 cps. Interval between pulses, 11 milliseconds. Slightly more harmonics than in single sound of some specimen.

Fig. 3. Single sound of specimen #5. Duration, 23 milliseconds; 5 cycles present; average repetition rate, 217 cps. Distorted wave form with at least 5 harmonics measurable.

Fig. 4. Double sound A of specimen #5. Total duration, 58 milliseconds. First pulse: 23 milliseconds, 5 cycles present, average repetition rate, 217 cps. Second pulse: 17 milliseconds, 4 cycles present, average repetition rate, 235 cps. Interval between pulses, 18 milliseconds.

Plate II

Fig. 5. Double sound B of specimen #5. Total duration, 48 milliseconds. First pulse: 26 milliseconds, 6 cycles present, average repetition rate, 231 cps. Second pulse: 13 milliseconds, 3 cycles present, average repetition rate, 231 cps. Interval between pulses, 9 milliseconds. Similar in wave form and harmonic content to the single sound.

Fig. 6. Double sound C of specimen #5. Total duration, 57 milliseconds. First pulse: 7 milliseconds, 2 cycles present, average repetition rate, 286 cycles. Second pulse: 33 milliseconds, 8 cycles present, average repetition rate, 243 cps. Interval between pulses, 17 milliseconds.

Fig. 7. Typical intermittent blast of *O. tau* during its breeding season in Narragansett Bay. Individual contacts usually repeated at intervals of 30 seconds or more.

Plate III

Fig. 8. Bimini toadfish with ventral body wall removed to show muscle-bordered air bladder *in situ*.

Fig. 9. Bimini toadfish after bisection of air bladder to show internal transverse septum.

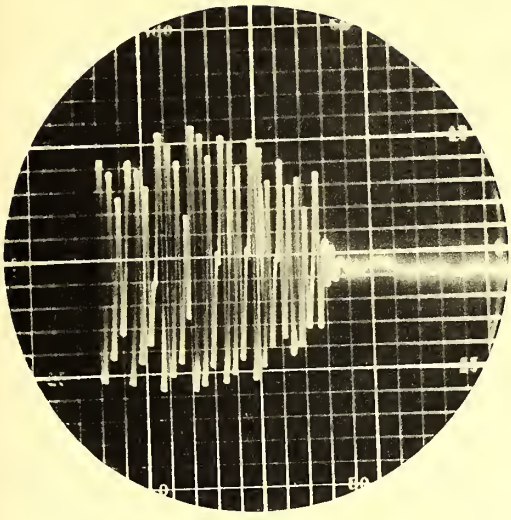


FIG. 1

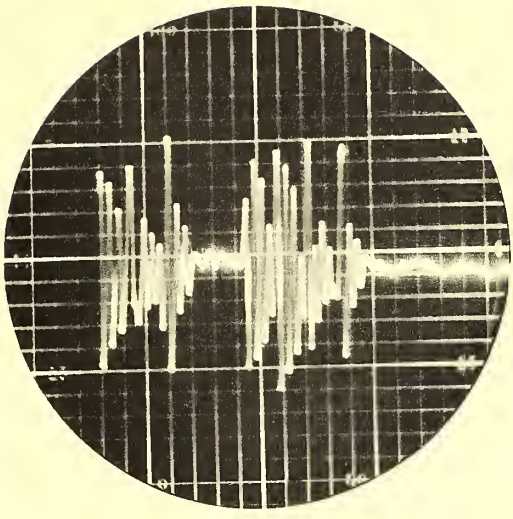


FIG. 2

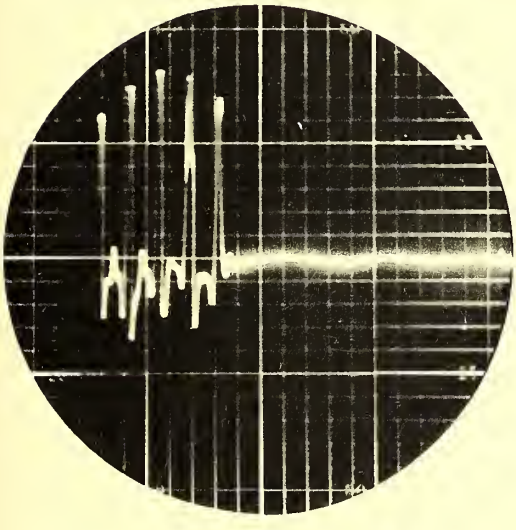


FIG. 3

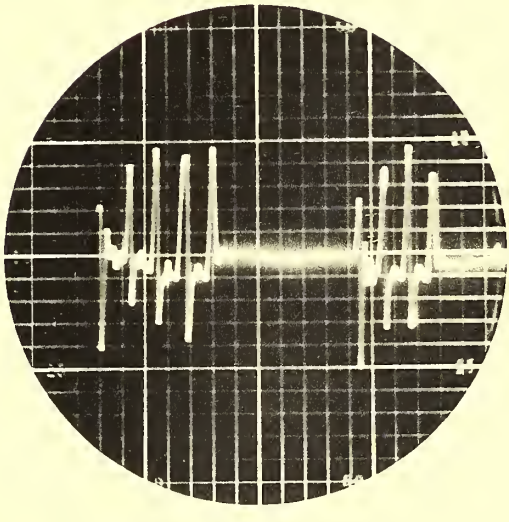


FIG. 4

THE PRODUCTION OF UNDERWATER SOUNDS BY OPSANUS SP.,
A NEW TOADFISH FROM BIMINI, BAHAMAS

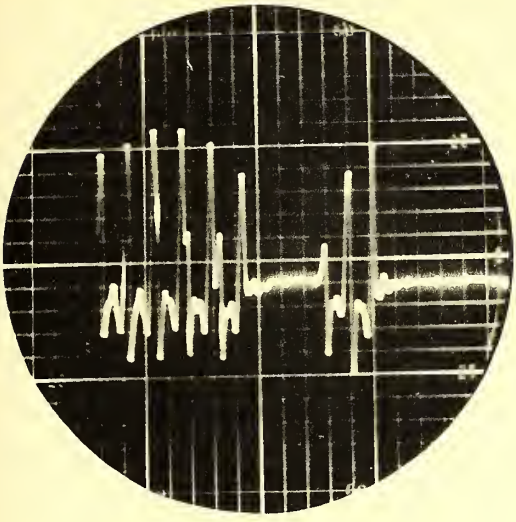


FIG. 5

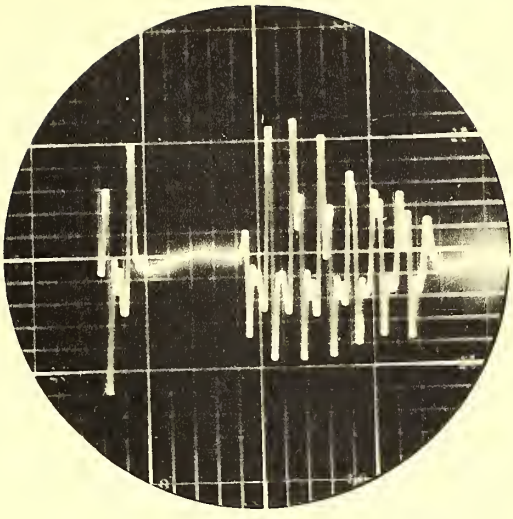


FIG. 6

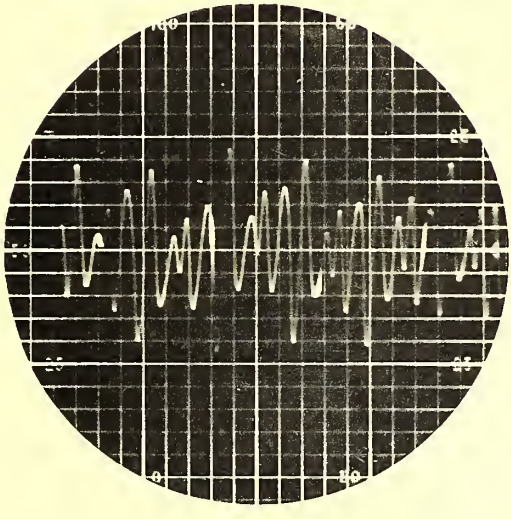


FIG. 7

THE PRODUCTION OF UNDERWATER SOUNDS BY OPSANUS SP.,
A NEW TOADFISH FROM BIMINI, BAHAMAS

FIG. 8

FIG. 9

THE PRODUCTION OF UNDERWATER SOUNDS BY OPSANUS SP.,
A NEW TOADFISH FROM BIMINI, BAHAMAS

5

Some Aspects of the Behavior of the Blennioid Fish *Chaenopsis ocellata* Poey[1]

C. Richard Robins, Craig Phillips & Fanny Phillips

The Marine Laboratory, University of Miami, and the Miami Seaquarium, Miami, Florida

(Plates I-III; Text-figure 1)

Introduction

THREE individuals (two males and a female) of *Chaenopsis ocellata* Poey, the pike blenny, were captured during January, 1958, and were placed in an aquarium. Studies were conducted both at The Marine Laboratory and the Miami Seaquarium. During the course of the study the two males were killed by crabs inadvertently introduced into the tank, but each was replaced within a few days. One male measured 73 mm. in standard length; the other males were about the same length and the female was about 10 mm. smaller. Detailed notes were recorded at irregular intervals for six months and more casual observations were made nearly every day.[2]

Longley (Longley & Hildebrand, 1941: 275) described *C. ocellata* in some detail and recorded some aspects of its life history. However, certain details of coloration pertinent to the present study were not noted. The dorsum is sand-colored and is crossed by a variable number of dark bars which merge into the very dark sides. Although our specimens were kept in dark-bottomed aquaria and then on pale sand for many weeks, no change was seen in the sharp contrast between the pale dorsum and the dark sides. This differs from the account of Longley & Hildebrand (1941:275). Perhaps *C. ocellata* is another example of a background-contrasting species (see Breder, 1949: 93-94), but more likely the ability to change its general coloration has been lost as a consequence of life in tubes and holes. As noted below those features of *C. ocellata* that change are anterior on the body, i.e., on the portion normally exposed.

Males have the spinous portion of the dorsal fin dusky, the color intensified when the fish is disturbed. A prominent black comma-shaped mark is present on the membrane between the first two dorsal spines. In larger males the black mark is bordered on the first spine by an azure streak. The black comma partially encloses an orange spot and in some individuals the two are separated by a white streak (Plate II, Figs. 5, 6). The branchiostegal membranes, which appear dark in normal folded position, are azure and very conspicuous when fanned out. The oral cavity is milky and the iris is orange.

Females are similarly bicolored on the body but lack any color in the dorsal fin other than scattered dark flecks along the spines and rays. The branchiostegal membranes are dark but not tinted with blue.

Pectoral and pelvic fins are colorless in both sexes but the anal fin is somewhat speckled along the rays.

Three preserved specimens from The Marine Laboratory of the University of Miami (UMML) were available for study; numbers in parentheses refer to the number of specimens and their standard length in millimeters: UMML 2320 (1, 61): dorsal rays XXI + 32, anal rays II + 35, pectoral rays 13-13, striated caudal rays 13. UMML 2376 (1, 73 + one mutilated specimen): dorsal rays XVIII + 34, anal rays II + 34, pectoral rays 13-13, striated caudal rays 13.

[1]Contribution No. 230 from The Marine Laboratory, University of Miami.

[2]This study was supported by a National Science Foundation grant-in-aid (NSF-G-3881) which the senior writer gratefully acknowledges. John E. Randall and Gilbert L. Voss of The Marine Laboratory and Charles M. Breder, Jr., of the American Museum of Natural History have kindly reviewed the manuscript and offered numerous suggestions. We are especially indebted to Walter R. Courtenay, Jr., of The Marine Laboratory for his patient work in securing the photographs, to Gilbert L. Voss for identifying the annelid worms and to Conrad Limbaugh of Scripps Institution of Oceanography for providing observations on a related species, *Chaenopsis alepidota* (Gilbert).

UMML 2319 (1, 67): dorsal rays XIX + 33, anal rays II + 35, pectoral rays 13-13, striated caudal rays 13. These data agree well with counts listed in a recent review of the genus by Böhlke (1957: table III).

Chaenopsis ocellata is known from southeastern Florida (Biscayne Bay at Miami to the Dry Tortugas) to Cuba. Two specimens (UMML 2377) recently collected in the Virgin Islands may represent an undescribed species.

Observations

Resting Behavior.—In normal resting position the pike blenny lies on the bottom with its body in a sine or simple curve (Plate I, Figs. 2, 3). The head is raised sharply and swung from side to side as other fish or invertebrates attract its interest. The foreparts are slightly elevated and are supported by the pelvic fins. In resting position the dorsal fin is fully depressed, the transparent pectorals are spread, held stationary or fanned slowly and aid in supporting or steadying the body. In its normal habitat, however, it is doubtful whether *Chaenopsis* is often found in the open. Longley & Hildebrand (1941: 275) state that it inhabits tubes which may be built by the fish itself. Walter R. Courtenay, Jr., (personal communication), observed one in a hole under a rock at Soldier Key, Florida.

For more than a month the blennies lived in an aquarium with an ample supply of sand and food. No tubes were constructed nor did the fish make any effort to burrow. Tubes of the terebellid worm, *Loimia medusa* (Savigny), were placed in the aquarium and were immediately occupied by the pike blennies. The blenny would slowly approach the tube and peer directly into the opening, which was about 20 mm. in diameter. The approach was always directly toward the opening, never from the side. If the tube was empty, the dorsal fin was kept depressed and the blenny reversed its position with its head about one-third to one-half of the body length in front of the opening. The body was then drawn up in a series of curves until the caudal fin was about at the level of the tube opening. Entrance into the tube, always tail first, is like the action of a person groping blindly for some object behind him. Here the caudal fin does the groping until contact is made with the tube, at which time the blenny uncurls and slides backward into the tube. This action rarely accomplishes its objective on first try. More often, the blenny ends up tight against the outside of the tube. After a few seconds of rest it suddenly seems aware that all is not well, at which time the entire act, starting with the eyeing of the tube entrance, is repeated until the attempt is successful; on one occasion six tries were required. After a first unsuccessful effort the blenny may peer into the tube from the side rather than make an entirely new approach, although this too may be done, especially if the blenny is distracted in the interim.

Although the tubes of *Loimia medusa* are those that appear in the accompanying illustrations, tubes of the onuphid worm, *Onuphis magna* (Andrews), were also used extensively in the later portions of this study, since they are abundant in the pike blenny's habitat. Tube diameters of *Loimia* average slightly less than 20 mm. Those of *Onuphis* are a few millimeters narrower and of more rigid construction. Empty tubes were normally presented but one tube of *Loimia* containing the worm was placed in the tank. A pike blenny evicted the worm, but little importance may be attached to this incident since the tube was lying flat on the sand with both ends exposed. No differences were noted in the behavior of the pike blenny with the two types of worm tubes. Artificial tubes of rubber and glass were employed without success, although one blenny briefly entered a glass tube whose aperture was flush with the substrate.

Sand at one end of the tank was elevated to support the worm tubes (*Onuphis*) in a vertical position. The blennies approached them directly as before but had greater difficulty in backing into them if the openings were more than one inch from the surface of the sand. The ends of the vertical worm tubes encountered in the grassy areas where the blennies were obtained are generally two or three inches above the surface of the sand and are curved so that the angle of the aperture is parallel with the sand, or nearly so. In the same area are broken tubes of various lengths which lie on the bottom. If given a choice of a horizontal or a vertical tube whose aperture is more than an inch above the surface of the sand, the blennies generally chose the horizontal tube. When the vertical tubes were nearly flush with the bottom, no particular preference was indicated. If the tube aperture were high enough to cause the blennies difficulty, they occasionally tugged at the rim with their jaws in an apparent attempt to pull it closer to the substrate.

Resting position in a tube does not vary markedly from that noted above except that the portion of the body within the tube must be straight. Usually the entire head shows and is canted upward. Often the body is extended from a horizontal tube until the pelvic fins are free and can act as braces against the bottom to elevate the body (Plate II, Fig. 7). In vertical

tubes, the blennies still cant their head upward from the horizontal opening at a 30° to 45° angle. Generally the body is extended until the pelvic fins are free, although here they are too far from the bottom to serve as braces.

Respiration by the resting pike blenny is slow and not conspicuous; i.e., the mouth is not gaped and only a small upper portion of the gill opening is utilized. As noted above, the pectoral fins usually are held motionless and braced against the bottom but are fanned, apparently for stability, if the blenny raises off the bottom.

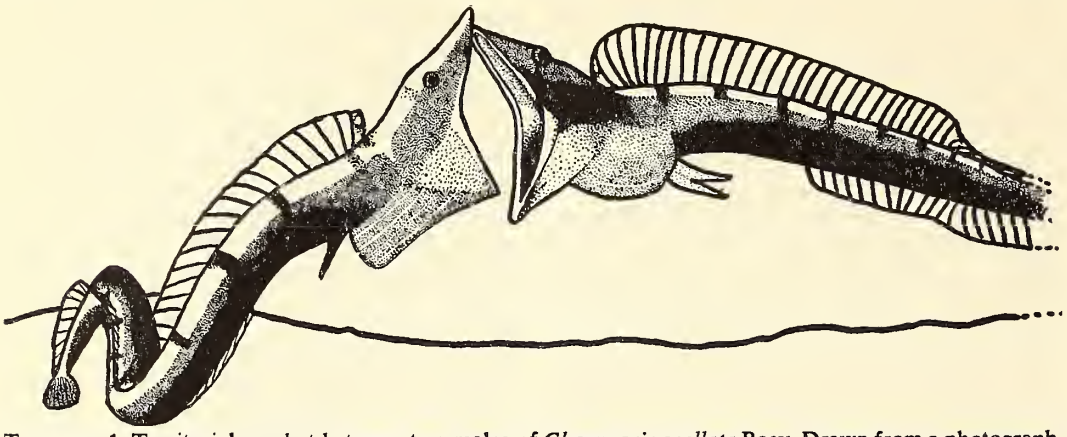
The blennies were never observed to burrow in the sand or to hide under objects. When resting on open sand the fanning pectorals create a broad, shallow depression about the blenny. If the tube entrances are blocked with debris, the blenny will push its head through and reconnoiter for a few seconds. Then it moves farther forward and vigorously fans the pectorals and sometimes also the pelvics. The sand is swept away and larger objects are shoved aside with the snout and side of the head.

Threat Behavior.—The approach of any animal or even drift material within about ten inches excites the interest of the pike blenny. Its head is raised and the dorsal fin, except for the first several spines, is erected (Plate I, Fig. 2). A slight increase in the respiratory rate is observed and the dorsal fin and head may darken slightly. As the organism drifts by or swims off the dorsal fin is lowered. Closer approach by a second pike blenny results first in the interest behavior noted above and then, if further approach is made, in threat. The transition is marked by a rapid increase in the respiratory rate, an intense darkening of the spinous portion of the dorsal fin and of the head, spreading of the pectoral fins and finally by a wide gaping of the mouth and spreading of the azure branchiostegal membranes (Plate II, Fig. 4). Gaping as an expression of threat is well known in fishes. Walters & Robins (*In Press*) noted differences in coloration of the oral cavity in two species of toadfish. The pike blennies may afford a similar example, for the oral cavity of *C. ocellata* is pale, that of *C. alepidota* black. Threat display is never followed directly by an attack. In most instances, especially where the intruder was smaller, the warning display sufficed to deter its approach. No instance of deferred combat as described by Raney (1947: 127) or Reighard (1910: 1128) for cyprinid fishes was seen in *Chaenopsis*.

The approach of a female pike blenny was usually ignored by the male. The female, according to Longley & Hildebrand (1941: 275) is readily distinguished by its lower and paler spinous dorsal fin and in details of coloration already described.

The large supralateral eyes of the blenny may be rotated and tilted with remarkable freedom. From its behavior, the writers conclude that this blenny depends largely on sight in its activities.

Attack Behavior.—Continued approach of a pike blenny results in aggressive behavior. Once triggered, the attack is carried to completion even if the intruder is removed. Both blennies exhibit rapid respiration with slight and rapid opening and closing of the mouth. The branchiostegals are slightly spread and the dorsal fin is fully erected. The orange and blue area between the first two dorsal spines is exposed (Plate III, Fig. 11) and by twisting the first two spines laterad of the others this color mark is directed forward and toward the intruder (Plate II, Fig. 6). To this point both blennies show the same behavior except that the intruder does not exhibit the prefatory threat cycle (Plate II, Fig. 4). If the intruder approaches rapidly, the defending male goes directly into attack behavior. The two blennies meet snout to snout and then raise the anterior two-thirds of their bodies well off the substrate, the tails being curled on the bottom for support. The mouths are gaped enormously, in contact with each other, the branchiostegal membranes spread fully and the pectorals fanned rapidly to maintain position (Text-fig. 1). If the combatants are nearly equal in size, the two may rise and fall in combat several times, never losing their oral contact. A smaller male is usually subdued rapidly on the first rising contact, but here it must be noted that a much smaller male, unless it is the defendant, is usually discouraged by the earlier warning display. In the aquarium a small male was forced into combat, a situation that presumably would not occur in nature, by moving one blenny into the territory of the second, usually with a probe or plate of glass. The winning male is the one that suddenly shifts its mouth sideways across the other's and clamps down hard. At this point the defeated male folds his dorsal fin and branchiostegal membranes (Plate II, Fig. 5) and contact is broken as both males drop to the bottom. Immediately the defeated male resumes the normal slow respiratory rate and after a few seconds retreats. The victor maintains rapid breathing and keeps the dorsal erected (Plate I, Fig. 1; Plate II, Fig. 5) but the branchiostegal membranes are folded. No additional attack is made on the defeated blenny even though it may remain nearby for a few seconds. Return to resting behavior on the part of the victor is not accomplished for several



TEXT-FIG. 1. Territorial combat between two males of *Chaenopsis ocellata* Poey. Drawn from a photograph.

minutes, although the color spot in the dorsal fin is covered after a few seconds. Unlike the behavior of other fishes (Raney *et al.*, 1953:99), the defending pike blenny apparently does not have an advantage over the intruder; the winner is determined by size and aggressiveness alone. The pike blenny defending a worm tube is in quite a different position and is seldom displaced by a larger aggressor except as noted later. Attack from the shelter of a worm tube does not differ from that described above. The defending blenny does not completely leave the tube, however, unless beaten. In some instances the defending blenny would be doubled back over the worm tube when fighting off an intruder. A male blenny would tolerate a female in the other end of the tube (i.e., a horizontal tube) since it was long enough to accommodate both. In one instance the second male occupied the other end of the tube for a few moments.

The pike blenny readily abandons its tube when outside pressure is applied. By this method we chased the larger male from his home and allowed the second and smaller male to enter. The original male was then returned to the tank. Although a second and empty tube was available he returned to the original tube and when the second blenny was sighted therein, displayed the attack pattern. The second blenny had retreated into the tube so that only the tip of its snout showed. The attacker raised his body on the pelvic fins, erected the dorsal fin and directed the orange dorsal spot toward the tube opening. The first two or three spines may be twisted to either side in directing the flash spot forward. When the second male made no effort to join the fight, the attacker swam to the tube, the motion being best described as a strike from a coiled position, and very snake-like. Its mouth was opened wide, the tip of the lower jaw on the sand and the upper jaw well above the tube. The dorsal fin and branchiostegals were still broadly displayed. Next the mouth was clamped suddenly and strongly down over the snout of the "defending" blenny, the action resulting in a partial folding of the branchiostegal membranes. Plate III, Fig. 8, shows the action nearly completed. The defending blenny erupted from the tube, speeded by several snaps of the aggressor (Plate III, Fig. 9), and fled over the tube to the far end of the aquarium. Such rapid swimming is accomplished by anguilliform movements with the vertical fins depressed. After a minute the victor entered the tube, employing the behavior described earlier. In some instances the defending male remained completely in the tube, at which time the attacker yanked several pieces from the tube entrance and pressed its attack into the tube with the same end result.

Other species of fishes elicited varied responses. Two common grass flat inhabitants, *Callionymus calliurus* Eigenmann & Eigenmann, and a species of *Syngnathus* were never attacked or threatened, while juveniles of *Sparisoma,* equally common on the grass flats, were vigorously attacked and snapped at. At no time did they return the fight.

Behavior Before a Mirror.—A pocket mirror was placed in front of the tube occupied by a male pike blenny. The type of response was controlled by the intervening distance and the rapidity with which the mirror was advanced. At 10 inches interest was exhibited. At about six inches, interest gave way to threat. Failure to remove the mirror at this point did not result in attack. Approach to a point somewhat less than the body length of the blenny resulted in attack. Slow approaches were successful in a closer placement of the mirror. A fast approach alarmed the blenny and resulted in immediate attack responses. Attack on the mirror image was violent and since the blenny was evidently

neither victorious nor defeated the attack was repeated many times. Plate III, Fig. 11, shows the initial attack of a sequence in its early phase, Plate III, Fig. 10, a momentary pause before a second attack. The blenny which is in the middle of combat (in this case between repeats) is much darker in dorsal-fin and head coloration compared to the same fish (Plate III, Fig. 10) at the start of combat (see also Plate III, Fig. 8). In combats between two blennies the issue was decided in every instance during the initial attack and repeated attacks did not occur.

Proximity of Tubes and Behavior.—Two occupied tubes were placed in the same section of the tank, both occupied by males of *Chaenopsis*. Again, threat was exhibited at a distance of about six inches. If the tubes are left in this position, threat display usually subsides but may be resumed if one of the blennies moves suddenly. Gradually the two appear to accept the reduction in territory size and threat behavior ceases. Placement of the tubes at a point where the two blennies may easily reach each other results in immediate combat. In one instance the defeated blenny retreated so rapidly into the tube (*Loimia*) that the side was broken out, whereby the vanquished fish escaped.

Obviously the pike blenny is a strongly territorial fish but we can report nothing on its territory size in nature. Efforts to observe pike blennies in the vicinity of Miami where the study material was obtained have not been successful. The concentration of individuals would appear to be very low in the region. Similarly, at Soldier Key where one specimen was collected and a second observed (see above), a large poison station yielded a variety and abundance of small bottom fishes but no pike blennies.

Threat and attack may be elicited by extraneous objects such as a pencil or finger. Attack is not repeated on such objects and after several trials no response will be given for one or several days. The initial attack, however, does not lack in vigor and one blenny was completely raised from the water before loosening its grip.

Spurious Attack.—While photographing the pike blennies, attacks were stimulated repeatedly for an hour or more at a time without diminution of the response though it became more difficult to prod one blenny into the second's territory. The intrinsic factors that control the various responses are apparently maintained at a high level in the male pike blenny and accumulate if no need for their use is forthcoming. A pike blenny kept alone or far removed from another pike blenny may vary its behavior suddenly. Thus its head and dorsal fin will darken periodically and then fade, without any external stimulus. Attack usually will follow several such changes. Since no fish or invertebrate is near, the attack is directed against some nearby object such as a small stone or merely a nearby point in the sand.

Feeding Behavior.—Feeding was observed and recorded when the blennies were free in a 15-gallon aquarium and when they were in both horizontal and vertical tubes. Any drifting or swimming object elicited interest. At such times the body, if relatively straight at the time, was now curled and the dorsal partly erected (Plate I, Fig. 2). A quarter-inch grass shrimp (*Tozeuma*) was caught by a sudden strike from the semi-coiled position. Shrimp were caught from the side, vigorously clamped and then shifted longitudinally in the mouth, and after several bites swallowed entire. If the grip was not satisfactory the shrimp was spit forward and a fresh grip made. The dorsal flash spot was not exposed, nor were the branchiostegal membranes. Food items never elicit threat display. On one occasion strikes were directed against three small shrimp which swam by above the bottom, and a fourth shrimp which rested nearby on the sand was stalked and caught. The pike blenny will readily leave its tube in catching food. A small mojarra (*Eucinostomus*) about 12 mm. long was caught and eaten and a small piece of ground fish placed nearby was also eaten.

Observations on *Chaenopsis alepidota* (Gilbert)

A related species, *C. alepidota*, occurs in the eastern Pacific in the Gulf of California. Böhlke (1957: 99-102) recently described a new subspecies, *C. a. californiensis,* from Santa Catalina Island, restricting the nominate form to the Gulf of California. In addition to geographical considerations, two specimens forwarded by Conrad Limbaugh were studied, and the following observations may be allocated to *C. a. alepidota.* Identification of the tubes with *Chaetopterus* is doubtful. The tube diameter is about 20 mm.

Mr. Limbaugh, of the Scripps Institution of Oceanography, has generously permitted the writers to include the following notes recorded by him, Andreas Rechnitzer and Earl A. Murray.

"Small tube fish were observed in tubes off Los Angeles Bay in a protected cove on one of the many small islands located about a mile from the shore during September 1953.

"These slender mottled brown fish occupied parchment-like tubes resembling those of the worm *Chaetopterus*, which protrude from the sand and open toward the surface. A few occupied small holes in the sand. These tubes and

holes were located on a sloping coarse shell and coraline sand bottom in a very protected rocky cove. The bottom was partially covered with short brown algae and scattered outcrops of volcanic rock projected through the sand. The estimated water depth varied between 8 and 16 feet, depending on the tide.

"As a skin diver approached the tube, the fish would withdraw into the tube, but if the diver attempted to cover the tube, the fish would leave and seek another. They entered the tubes or holes tail first.

"Several specimens were captured by placing a glass jar over the tubes. The lengths were estimated to be 3-4½ inches."

Additional observations on *C. a. alepidota* were recorded by Ron Church and Mr. Limbaugh.

"Adult tube fish were observed in tubes off the protected point of San Luis Gonzaga Bay during July, 1956. A group of adults, some in breeding color, occupied parchment-like tubes, possibly those of the worm *Chaetopterus,* which projected above the sloping sand bottom in a very protected area. Some of the occupied tubes extended as much as 3 inches above the substrate. The depth varied between 10 and 18 feet, depending on the tide. The bottom was of coarse sand, much of which was covered by a growth of brown algae.

"The larger fish were brightly marked, but were able to control the intensity of the pattern. They intensified the colors and increased the contrast when they threatened their neighbors or the photographer.

"The threatening behavior consisted of a rapid bobbing up and down while the head was in a horizontal position. The fins and throat were expanded and the mouth with its black interior was held open. Sometimes they would leave their tubes after they had worked themselves into a rage and lock jaws with their neighbors. They would spin a few times and return to their tubes. This same behavior was repeated if one of their neighbors, interested in catching a mysid, wandered into their territory.

"Feeding consisted of snapping up some of the many mysids which swam in clouds over the bottom. Usually the fish merely extended itself to catch one, but sometimes would leave the tube to follow one.

"If the observer covered the tubes with sand, the fish would probe its head through the sand and fan the sand out of the area by a rapid movement of its pectoral which sent the sand forward. Larger pieces of gravel were picked up with the mouth and carried a few inches away. The tube fish were estimated to range in size between 4 and 5½ inches."

On May 28, 1958, Mr. Limbaugh again checked the population at Los Angeles Bay.

"Adults and sub-adults of *Chaenopsis* were found to be abundant in a bay on the west side of Isla Ventana in the Gulf of California just outside of Los Angeles Bay, Baja California.

"The bottom had changed considerably. It was more cobble than sand and had a healthy growth of plants and other organisms in contrast to the relatively barren sand observed there on my first visit (see above).

"Tube fish were abundant, most of them in parchment-like worm tubes between cobbles but some were swimming free. Several sub-adults were observed fighting. A few adults showed breeding color but no eggs were found in their tubes. One large adult (presumably a female) was very heavy with a swollen yellow belly. The population occurred in depths of about 3 feet to 20 feet."

Examination of color photographs provided by Mr. Limbaugh shows that *C. alepidota* has color markings in the same locations as does *C. ocellata.* Presumably they play similar roles in the behavior of *C. alepidota.* Details of coloration differ markedly between the two species. The spot in the spinous dorsal is located between spines 1 and 2 but is black, narrowly margined above by a pale area which may be orange. Most of the branchiostegal and gular region is black except for the white tip of the lower jaw. The outer corners of the mouth are bright orange whereas the cheek is white except for a dark blotch, about equal in size to the eye, on its posterior portion. The upper branchiostegal region and lower preopercle are bright orange; the color is not continuous with the orange at the corner of the mouth. The orange is set off from the black below, and above, by a well defined white streak. The opercle and postorbital region are largely black, the pigment forming two large blotches, each wider than the length of the snout. The orange projects for a short distance dorsad between the two blotches. The opercular membrane is white.

More detailed observations are needed both of *C. ocellata* and *C. alepidota* to determine whether differences, such as the absence of bobbing behavior in *C. ocellata,* really exist in nature.

Summary

Behavioral observations are described for the pike blenny, *Chaenopsis ocellata* Poey, under aquarium conditions. The species is strongly

territorial and well suited for aquarium study. Resting, threat, attack and feeding patterns are discussed. A multicolored spot in the spinous dorsal fin and the azure branchiostegal membranes play important roles in threat and attack behavior, as does the erection of the dorsal fin, gaping of the mouth and a change in respiration rate.

Some notes are provided for *Chaenopsis alepidota* (Gilbert), a related species from the eastern Pacific, but detailed comparison of the two species is not yet possible.

Bibliography

Böhlke, James E.

1957. A review of the blenny genus *Chaenopsis*, and the description of a related new genus from the Bahamas. Proc. Acad. Nat. Sci. Philad., 109:81-103, 3 figs., pls. 5-6.

Breder, Charles M., Jr.

1949. On the relationship of social behavior to pigmentation in tropical shore fishes. Bull. Amer. Mus. Nat. Hist., 94(2):83-106, 2 figs., pls. 3-10.

Longley, William H. & Samuel F. Hildebrand

1941. Systematic catalogue of the fishes of Tortugas, Florida with observations on color, habits, and local distribution. Carn. Inst. Washington, Pub. 535, Papers from Tortugas Lab., 34: xiii + 331, 34 pls.

Raney, Edward C.

1947. *Nocomis* nests used by other breeding cyprinid fishes in Virginia. Zoologica, 32(3): 125-132, pl. 1.

Raney, Edward C., Richard H. Backus, Ronald W. Crawford & C. Richard Robins

1953. Reproductive behavior in *Cyprinodon variegatus* Lacépède, in Florida. Zoologica, 38(2): 97-104, 2 pls.

Reighard, Jacob

1910. Methods of studying the habits of fishes, with an account of the breeding habits of the horned dace. Bull. U. S. Bur. Fish., 28 (1908), Pt. 2: 1111-1136.

Walters, Vladimir & C. Richard Robins

(*In press*). A new toadfish (Batrachoididae), considered to be a glacial relict in the West Indies. Amer. Mus. Novitates.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. A victorious male *Chaenopsis ocellata* Poey immediately after combat. Note the fully erect dorsal fin, the partially folded branchiostegal membranes and the rapid respiratory rate, indicated here by the open mouth.

Fig. 2. A male *C. ocellata* in resting position on an open sand bottom. The partially erect dorsal fin is evidence of interest in some approaching object or animal.

Fig. 3. A male *C. ocellata* in normal resting position on an open sand bottom as viewed from above.

Plate II

Fig. 4. Threat display by one male *C. ocellata* toward a second approaching male. Note the gaping mouth, the spread and rigid pectorals and the folded anterior portion of the spinous dorsal fin.

Fig. 5. End of combat between two males of *C. ocellata*. The erected dorsal and open mouth of the fish on the left indicates the victor, the closed mouth and folding dorsal fin of the fish on the right marks the defeated fish, which will shortly move off.

Fig. 6. Combat about to be broken off between two males of *C. ocellata*. Again the folding dorsal fin on the right-hand fish signifies defeat. Note especially the use of the pelvics as a brace against the bottom and the manner in which the dorsal flash spot is directed forward.

Fig. 7. Resting behavior by a male *C. ocellata* in a terebellid worm tube (*Loimia medusa*). The dorsal fin is folded and the pectorals are fanned for stability.

Plate III

Figs. 8 & 9. Attack by a male *C. ocellata* on a second male which usurped the tube of the attacking fish during a momentary absence. In Fig. 8 the male is about to bite the snout of the second male and in Fig. 9 the second male is in full flight. Fig. 8 shows the end of a strike from a coiled position.

Figs. 10 & 11. Behavior of a male *C. ocellata* toward a mirror image. Fig. 11 shows the initial attack display, Fig. 10 momentary pause before a subsequent attack. Note the intense darkening of the head and dorsal fin of the male in Fig. 10 after one attack.

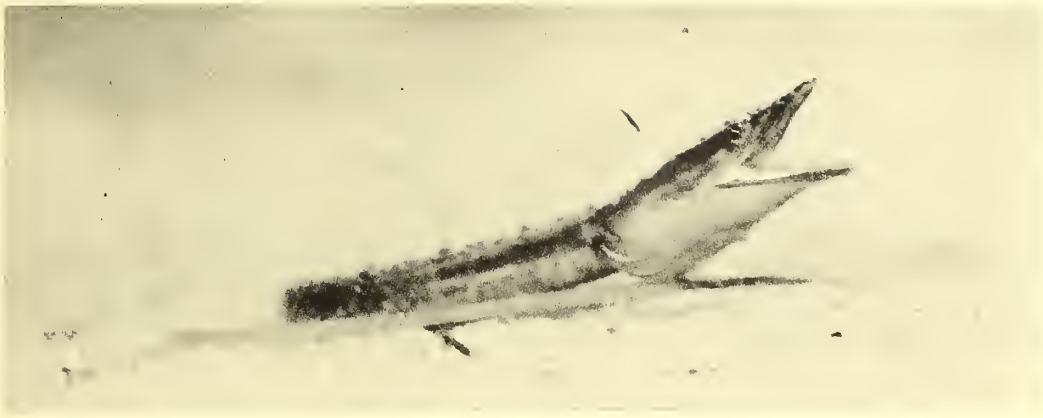


FIG. 1

FIG. 2

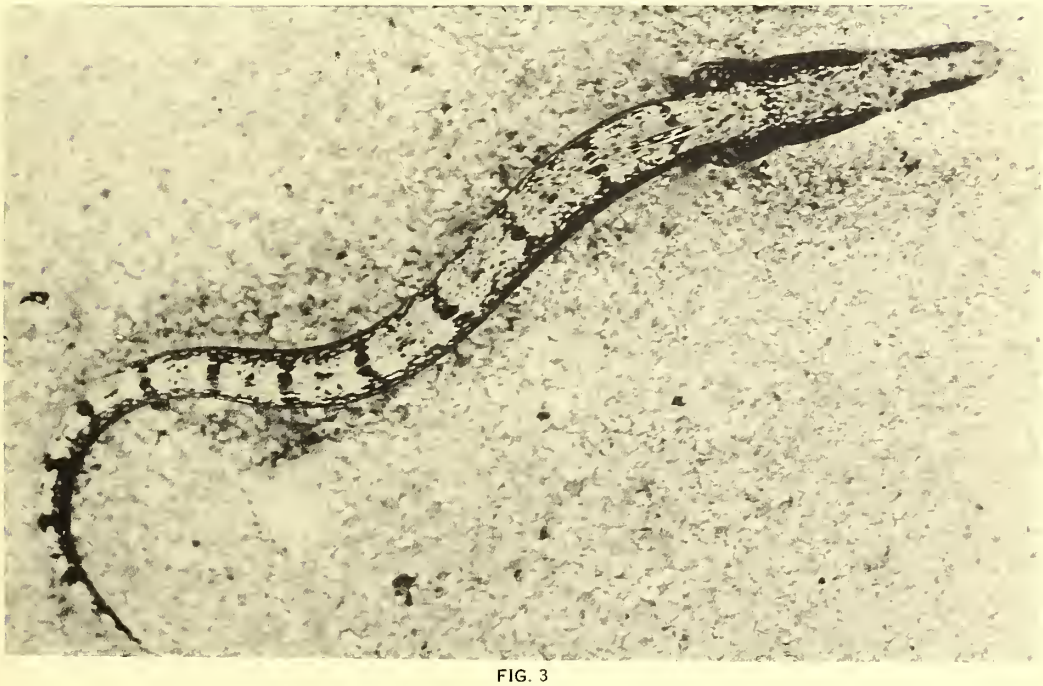


FIG. 3

SOME ASPECTS OF THE BEHAVIOR OF THE BLENNIOID FISH
CHAENOPSIS OCELLATA POEY

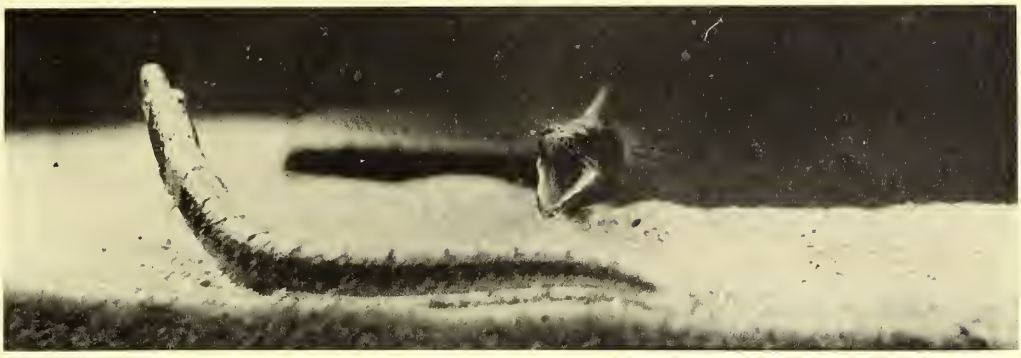


FIG. 4

FIG. 5

FIG. 6

FIG. 7

SOME ASPECTS OF THE BEHAVIOR OF THE BLENNIOID FISH
CHAENOPSIS OCELLATA POEY

FIG. 8

FIG. 9

FIG. 10

FIG. 11

SOME ASPECTS OF THE BEHAVIOR OF THE BLENNIOID FISH
CHAENOPSIS OCELLATA POEY

NEW YORK ZOOLOGICAL SOCIETY

GENERAL OFFICE
30 East Fortieth Street, New York 16, N. Y.

PUBLICATION OFFICE
The Zoological Park, New York 60, N. Y.

OFFICERS

PRESIDENT	VICE-PRESIDENTS	SECRETARY	TREASURER
Fairfield Osborn	Alfred Ely Laurance S. Rockefeller	George Wall Merck	David H. McAlpin

SCIENTIFIC STAFF:

John Tee-Van ..... *General Director*

James A. Oliver... *Director, Zoological Park*

Christopher W. Coates.. *Director, Aquarium*

ZOOLOGICAL PARK

Grace Davall..... *Assistant Curator, Mammals and Birds*

Joseph A. Davis, Jr... *Assistant Curator, Mammals*

William G. Conway.. *Curator, Birds*

James A. Oliver.... *Curator, Reptiles*

Charles P. Gandal .. *Veterinarian*

Lee S. Crandall..... *General Curator Emeritus*

William Beebe..... *Honorary Curator, Birds*

AQUARIUM

James W. Atz..... *Associate Curator*

Carleton Ray..... *Assistant to the Director*

Ross F. Nigrelli..... *Pathologist & Chairman of Department of Marine Biochemistry & Ecology*

C. M. Breder, Jr..... *Research Associate in Ichthyology*

Harry A. Charipper... *Research Associate in Histology*

Homer W. Smith..... *Research Associate in Physiology*

GENERAL

William Bridges .. *Editor & Curator, Publications*

Sam Dunton ..... *Photographer*

Henry M. Lester.. *Photographic Consultant*

DEPARTMENT OF TROPICAL RESEARCH

William Beebe..... *Director Emeritus*

Jocelyn Crane..... *Assistant Director*

David W. Snow ..... *Resident Naturalist*

Henry Fleming ..... *Entomologist*

John Tee-Van ..... *Associate*

William K. Gregory.... *Associate*

AFFILIATES

L. Floyd Clarke ..... *Director, Jackson Hole Biological Research Station*

SCIENTIFIC ADVISORY COUNCIL

A. Raymond Dochez
Alfred E. Emerson
W. A. Hagan
Caryl P. Haskins
John S. Nicholas

EDITORIAL COMMITTEE

Fairfield Osborn, *Chairman*

James W. Atz
William Beebe
William Bridges
Christopher W. Coates
William G. Conway
Lee S. Crandall
James A. Oliver
John Tee-Van

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 44 • PART 3 • DECEMBER 11, 1959 • NUMBERS 6 TO 8

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

6

The Ctenuchidae (Moths) of Trinidad, B.W.I. Part II. Ctenuchinae[1]

HENRY FLEMING

Department of Tropical Research,
New York Zoological Society, New York 60, N. Y.

(Plates I-III)

[This paper is one of a series emanating from the tropical field station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, British West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, and the annual rainfall is more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe, Zoologica, 1952, 37 (13): 157-184].

CONTENTS

INTRODUCTION

THIS is the second paper on the species of moths belonging to the Family Ctenuchidae that have been recorded in the literature from Trinidad or collected by the Department of Tropical Research of the New York Zoological Society at its biological station at Simla, Arima Valley, Trinidad.[2]

The present paper includes a key to the genera of the Ctenuchidae of Trinidad and keys to the species within the genera, as in the first part, which should be referred to for additional introductory detail. Part II includes photographs of the species collected at Simla, as an aid to biologists working on ctenuchids of the island. As in Part I, which dealt with the Euchromiinae, no attempt has been made to make complete references under the species of Ctenuchinae. References to the original description, pertinent or new synonymy, colored figures, helpful descriptions to the species or a specific reference to Trinidad have been cited.

Part I on the Euchromiinae contained 23 genera and 50 species, among them 9 species not previously reported from Trinidad and five that were described for the first time. Kaye & Lamont

[1]Contribution No. 992, Department of Tropical Research, New York Zoological Society.

[2]The first paper was: The Ctenuchidae (Moths) of Trinidad, B.W.I. Part I. Euchromiinae. Zoologica, 1957, 42 (10): 105-130.

(1927) and Lamont & Callan (1950) had reported 16 species of the Euchromiinae that we have not collected.

The present paper on the Ctenuchinae contains 23 genera and 60 species, among them two new species and 13 which are new locality records for Trinidad. The authors cited above collected 11 species of the Ctenuchinae which we have not collected.

The total number of ctenuchids recorded from Trinidad and included in Parts I and II is 110 species in 46 genera.

My thanks go to Miss Rosemary Kenedy, who made notes and took photographs of many of the holotypes of the ctenuchid species in the British Museum (Natural History) which aided in the determination of some of the species in question. Miss Kenedy also collected the greater part of the ctenuchid collection of the Department of Tropical Research. Thanks go also to Dr. William Beebe and Miss Jocelyn Crane for their part in assembling the collection and for advice and criticism. All photographs in Part I and in this paper were taken by Sam Dunton, Staff Photographer of the New York Zoological Society.

Key to Genera of Trinidadian Ctenuchidae

1. Hindwing with veins Cu_1 and Cu_2 stalked or united 2
   Hindwing with vein Cu_2 widely separated from vein Cu_1. If veins M_2 and M_3 are united, vein Cu_1 is close to or stalked with them 28
2. Vein M_2 of hindwing absent; wing fold or line of scales only present..... 3
   Veins M_2 of hindwing present; a distinct vein above lower cell angle..... 25
3. Abdomen wasp-like; constricted near base 4
   Abdomen not wasp-like..... 6
4. Forewing with vein R_5 arising distad of vein R_3 .....*Pseudosphex*
   Forewing with vein R_3 arising distad of vein R_5 5
5. Two dorso-ventral bladder-like processes at base of abdomen. No ventral valve in male .....*Pleurosoma*
   No bladder-like process at base of abdomen. Second and third ventral abdominal segments covered by a valve in male *Sphecops*
6. Forewing with vein R_2 stalked with vein R_1 .....*Psoloptera*
   Forewing with vein R_2 free, arising from cell 7
   Forewing with vein R_2 stalked with R_{3+4+5} 8
7. Forewing with veins M_2 and M_3 parallel for aproximately one-quarter the way to the margin of the wing. Hind tibia not fringed .....*Calonotus*
   Forewing with veins M_2 and M_3 immediately divergent and hind tibia fringed. *Macroneme* (part)
8. Forewing with veins M_1 to Cu_2 fringed with hair .....*Dixophlebia*
   Forewing otherwise 9
9. Forewing with accessory cell. Male with tufts on anal segment.....*Phoenicoprocta*
   Forewing without accessory cell and male without tufts on anal segment..... 10
10. Antennae with medial part of shaft distinctly dilated 11
    Antennae undilated or but little wider in some females 15
11. Scutellum of thorax with very long hair. *Homoeocera*
    Scutellum of thorax normal, essentially very short hair 12
12. Hind tibiae flattened with long scales on distal end and on tarsi..*Macroneme* (most)
    Hind tibiae and tarsi with normal scaling. 13
13. Hindwing with lower part of cell very short, the discocellular veins very oblique. *Isanthrene*
    Hindwing with the lower part of cell approximately half the length of the wing or longer 14
14. Forewing with vein Cu_2 straight. Facies lycid beetle-like .....*Dycladia*
    Forewing with vein Cu_2 curved...*Syntomeida*
15. Abdomen with some yellow, hindwings with some red, thorax with woolly hair, very large and robust insect.....*Histiaea*
    Otherwise 16
16. Forewing with vein M_2 distant from M_3 at origin 17
    Forewing with veins M_2 and M_3 approximate or connate..... 18
17. Hindwing with the lower discocellular vein very short .....*Loxophlebia*
    Hindwing with the lower discocellular vein a fifth the length of the middle discocellular vein .....*Mesothen*
18. Hindwing with the lower part of the cell less than a quarter the length of the wing .....*Pheia*
    Hindwing with the lower part of the cell more than a quarter the length of the wing 19
19. Forewing with vein R_1 stalked with radial veins .....*Chrostosoma*
    Forewing with vein R_1 free from cell.... 20
20. Forewing with vein M_1 connate with radial veins 21
    Forewing with vein M_1 from cell below radial veins 24
21. Forewing very narrow.....*Rhynchopyga*
    Forewing normal 22

22. Forewing with vein Cu_2 from near end of cell ..... *Nyridela*
Forewing with vein Cu_2 based on end of cell 23

23. Forewing with vein Cu_1 from lower angle of cell ..... *Leucotmemis*
Forewing with vein Cu_1 from above lower angle of cell ..... *Cosmosoma*

24. Distal end of hind tibiae and tarsal joints edged with scales ..... *Pseudomya*
Hind tibiae and tarsi unscaled ..... *Saurita*

25. Hindwing with veins Cu_1 and Cu_2 forked near margin of wing ..... *Horama*
Hindwing with veins Cu_1 and Cu_2 united.. 26

26. Outer half of forewing hyaline ..... *Amycles*
Forewing completely scaled 27

27. Anal segment of abdomen with lateral tufts ..... *Eriphioides*
Abdomen without anal tufts ..... *Ceramidia*

28. Hindwing with three veins from lower part of cell 29
Hindwing with four veins from lower part of cell 32

29. Forewing opaque 30
Forewing hyaline except for fine borders at margins of wing 31

30. Hindwing with outer margin convex, evenly rounded ..... *Delphyre*
Hindwing with outer margin concave, sinuate, produced at apical area and indented at cubital vein area ..... *Antichloris*

31. Outer margin of hindwing evenly rounded *Chrysostola*
Outer margin of hindwing with apex produced ..... *Urolasia*

32. Abdomen constricted at second segment.. 33
Abdomen not constricted 34

33. Inner margins of hindwing with a distinct lobe ..... *Trichura*
Inner margin of hindwing evenly rounded *Aethria*

34. Wings hyaline except for fine borders at margins of wings; most of abdomen with large lateral tufts of crimson-colored hair ..... *Dinia*
Wings usually fully scaled but may have small hyaline areas; abdominal tufts, if present, terminal 35

35. Palpi upturned with third joint continuing the line of direction 36
Palpi upturned or oblique with the third joint porrect, if oblique, beetle-like 44

36. Hindwing with vein M_2 arising close to or stalked with vein M_3 37
Hindwing with vein M_2 widely separated from vein M_3 40
Hindwing with vein M_2 and vein M_3 from cell 38

37. Hindwing with vein M_{2+3} from cell... *Euagra*

38. Forewing with hyaline band at apex of wing. Ithomiid-like species ..... *Agyrta*
Forewing with hyaline area, if present, at base of wing 39

39. Base of abdomen with rough hair, tympanic hoods inconspicuous. Forewing facies a complex series of spots ..... *Eucereum*
Base of abdomen smoothly scaled, tympanic hoods conspicuous. Forewing facies simple ..... *Napata*

40. Hindwing with vein R_s separating from vein M_1 beyond cell ..... *Cercopimorpha*
Hindwing with vein R_s from cell 41

41. Hindwing with vein M_3 and vein Cu_1 separating beyond cell 42
Hindwing with vein M_3 and vein Cu_1 from cell 43

42. Forewing with vein R_2 free from cell and with vein M_1 connate with radial branches ..... *Aclytia*
Forewing with vein R_2 stalked with radial branches and vein M_1 from below radial branches ..... *Heliura*

43. Facies of forewing containing a shade of red ..... *Cyanopepla*
Facies of forewing without red; dull brown or dull brown with white apex... *Episcepsis*

44. Middle and hind tibiae smooth. Forewings broad ..... *Ctenucha*
Middle and hind tibiae tufted at spurs. Forewings narrow, facies beetle-like... 45

45. Forewing with veins M_{2+3} from cell. Vein M_2 separating from vein M_{2+3}, one-quarter the distance from the cell to the margin of the wing. Minimum forewing expanse, 40 mm. ..... *Correbia*
Forewing with veins M_{2+3} separate or connate. Maximum forewing expanse, 32 mm. ..... *Correbidia*

Ctenuchinae

In this subfamily vein M_2 in the hindwing is never atrophied. In the instances in which vein M_2 and M_3 are forked or very occasionally completely fused, veins Cu_1 and Cu_2 are widely separated. In the Trinidad Ctenuchinae only *Euagra* has veins M_2 and M_3 on a long stem. Vein M_2 is widely separated from vein M_3 in *Horama* with veins Cu_1 and Cu_2 forked and in *Ceramidia* and *Amycles* with veins Cu_1 and Cu_2 fused. In *Eucereum* vein M_2 arises from the base or near the base of the short stem of veins M_3 and Cu_1.

Dinia Walker

The single species can be separated readily from other ctenuchid species of Trinidad by the carmine-colored lateral tufts along the flattened abdomen.

Dinia aeagrus (Cramer)
(Pl. II, Fig. 1)

Sphinx eagrus Cramer, 1779: 10, pl. 198, fig. C.
Dinia aeagrus, Hampson, 1898: 338, fig. 158.
Dinia mena, Hampson, 1898: 339.
Dinia aeagrus, Draudt in Seitz, 1915: 110, pl. 18c.
Dinia mena, Draudt in Seitz, 1915: 110, pl. 18c.
Dinia mena, Kaye & Lamont, 1927: 7.
Dinia aeagrus, Travassos, 1957: 188-205, 4 pl. & 48 figs.

Travassos (1957) has exhaustively described and discussed this species, with an abundance of figures and plates.

Material.—Ten males and three females.

Range.—Mexico to Argentina.

Trichura Hübner

The constricted abdomen gives the species of this genus the appearance of vespid wasps. Some species which possess a long anal appendage have been likened to ichneumonids.

1. Abdomen immaculate, dull iridescent blue ..... *fumida*
   Abdomen with iridescent spots..... 2
2. Abdomen with a sublateral series of white spots ..... *cerberus*
   Abdomen without sublateral series of white spots ..... *coarctata*

Trichura cerberus (Pallas)

Sphinx cerberus Pallas, 1772: 27, pl. 2, fig. 8.
Zygaena caudata, Fabricius, 1777: 277.
Cercophora urophora, Herrich-Schaeffer, 1855: 80, f. 266.
Trichura cerberus, Hampson, 1898: 342, fig. 160.
Trichura cerberus, Draudt in Seitz, 1915: 111, pl. 18d.
Trichura cerberus, Kaye & Lamont, 1927: 7.

This species has been reported by Kaye & Lamont from St. Joseph at the southern foot of the Northern Range, but we have not taken it at Simla.

In Hampson's key (1898: 341) he places this species in his first section of the genus, which is characterized by having an anal appendage in the males. However, the presence of this appendage is variable in collected specimens, at least from British Guiana.

Range.—Venezuela and eastern South America to southern Brazil.

Trichura fumida Kaye
(Pl. II, Fig. 2)

Trichura fumida Kaye, 1914: 115.
Trichura fumida, Draudt in Seitz, 1915: 112, pl. 18e.
Trichura fumida, Kaye & Lamont, 1927: 7.

The original description is based on a female. The characters of the male place it in the first section of the genus in Hampson's key (1898: 341) as practically every specimen collected possesses a long scaled appendage on the terminal segment. Except for this appendage, the white-fronted palpi, the white procoxae and some white on the remaining coxae, the male is similar to the female.

Material.—Nineteen males and six females.

Range.—Trinidad.

Trichura coarctata (Drury)
(Pl. II, Fig. 3)

Sphinx coarctata Drury, 1773: pl. 27, fig. 2.
Trichura coarctata, Hampson, 1898: 344, fig. 161.
Trichura coarctata, Draudt in Seitz, 1915: 112, pl. 18e.

Material.—Three males and three females.

Range.—Venezuela to southern Brazil. A new record for Trinidad.

Aethria Hübner

The carmine-colored abdominal anal tuft separates the two Trinidad species of this genus from other ctenuchids. The carmine-colored tufts in *Dinia* are along the sides of the abdomen. In *Phoenicoprocta vacillans* of the Euchromiinae the anal segment has two sublateral terminal tufts in the males. In *Phoenicoprocta* only the males have terminal tufts while in *Aethria* both sexes have them.

1. Dorsum of abdomen immaculate black except for terminal segment..... *carnicauda*
   Dorsum of abdomen black with iridescent blue or green maculation..... 2
2. Ventrum of abdomen with first three segments white followed by small sublateral white spots to white base of terminal tuft (male) ..... *aner*
   Ventrum of abdomen with large paired white spots at base (females)..... *jacksoni*

Aethria carnicauda (Butler)
(Pl. II, Fig. 4)

Eunomia carnicauda Butler, 1876: 400.
Aethria carnicauda, Hampson, 1898: 349 (in part).
Aethria carnicauda, Hampson, 1914: 221.
Aethria carnicauda, Draudt in Seitz, 1915: 114, pl. 18h.
Aethria carnicauda, Kaye & Lamont, 1927: 7.
Aethria carnicauda, Beebe, 1953: 155-159, pls. I, II.

The Trinidad form may represent a new subspecies or species, but until material is available from other localities it would be unwise to describe this form. The Trinidad material differs from typical *carnicauda* primarily in lacking blue spots on the dorsum of the abdomen.

Material.—Twenty-three males and 12 females.

Range.—Trinidad, Venezuela and Brazil.

Aethria aner Hampson
(Pl. II, Fig. 5)

Aethria carnicauda Hampson, not Butler, 1898: 349, pl. XII, fig. 9 (in part).
Aethria aner Hampson, 1905: 428.
Aethria aner, Hampson, 1914: 221, pl. XI, fig. 29.
Aethria aner, Draudt in Seitz, 1915: 114, fig. 18h.

The iridescent blue dorsal abdominal bands separate this species from *carnicauda.*

Material.—One male taken on January 28.

Range.—Described from Venezuela and a form, *auriflua* Draudt, has been described from French Guiana. *Aethria aner* is a new record from Trinidad.

Aethria jacksoni Kaye

Aethria jacksoni Kaye, 1924: 418, pl. XLV, fig. 6.
Aethria jacksoni, Kaye & Lamont, 1927: 8, pl. 2, fig. 6.

The description and figures of this species lead me to believe that it can hardly be anything else than the female of *Aethria aner.* I am not synonymizing *jacksoni,* only because as yet we have not collected any females of *aner* in Trinidad.

Range.—Described from one specimen collected in Trinidad.

Urolasia Hampson

Wasp-like moths with the basal segment of the abdomen constricted and with small hindwings. Vein M_2 united with vein M_3 in the hindwing.

Urolasia brodea (Schaus)
(Pl. II, Fig. 6)

Syntrichura brodea Schaus, 1896: 132.
Urolasia brodea, Hampson, 1898: 370, fig. 181.
Urolasia brodea, Draudt in Seitz, 1915: 123, pl. 19g.
Urolasia brodea, Kaye & Lamont, 1927: 8.

This species was described from Trinidad material and is also the species type. The genus contains two other species, *opalocincta* Druce from French Guiana, with white opalescent subdorsal abdominal bands, and *albipuncta* Druce from Venezuela, with dorsal and subdorsal white abdominal spots. Our Trinidad species, *brodea,* has metallic blue bands.

Material.—Fourteen males and two females.

Range.—Trinidad and British Guiana.

Chrysostola Herrich-Schaeffer

Our one species of this genus is a bright-colored small moth with the base of the abdomen hardly constricted. This genus can be separated from *Urolasia* by having vein M_{2+3} of the hindwing from above the lower angle of the cell rather than from the angle of the cell. This species is listed in Seitz under the genus *Abrochia.*

Chrysostola fulvisphex Druce
(Pl. II, Fig. 7)

Chrysostola fulvisphex Druce, 1898: 404.
Chrysostola fulvisphex, Hampson, 1898: 377, pl. XIII, fig. 13.
Abrochia fulvisphex, Draudt in Seitz, 1915: 125, pl. 19k.

The yellow-and-black-banded abdomen of this species is distinctive for this subfamily in Trinidad and will separate this species from other ctenuchinae.

Material.—Two males.

Range.—Panama to the Amazons. A new record from Trinidad.

Cercopimorpha Butler

This genus differs from the two preceding genera in having three median veins in the hindwing. However, vein M_3 is forked with vein Cu_1. Vein R_s is also forked with M_1.

Cercopimorpha dolens (Schaus)
(Pl. II, Fig. 8)

Heliura dolens Schaus, 1905: 191.
Cercopimorpha dolens, Hampson, 1914: 239, pl. XII, fig. 30.
Heliura dolens, Draudt in Seitz, 1915: 168.
Cercopimorpha dolens, Draudt in Seitz, 1915: 206, pl. 28n.

A brown moth with the area in the lower part of the cell and just below the cell semi-hyaline.

Material.—One male collected on January 2.

Range.—Described from Venezuela. A new record from Trinidad.

Episcepsis Butler

The dull brown, relatively unpatterned forewings of this genus are helpful in separating four of its species. The remaining species, *venata,* while having a pattern, nevertheless has fully scaled forewings. Species of *Eriphioides* and *Ceramidia* in Trinidad also correspond in having unpatterned wings, but the collar in *Episcepsis* is dull brown while it is iridescent in the other two genera.

The hindwing of the males of *Lenaeus* and the new species have a large anal lobe containing a long, bulky hair-pencil within the wingfold. This hair-pencil is usually conspicuous. The above two species are in Section I of Hampson (1898: 385). On the other hand, the males of *hypoleuca, redunda* and *venata* have the tornus of the hindwing only slightly produced and the hair-pencil is frequently hidden within the wingfold. These three species are in Section II of Hampson (1898: 386).

1. Forecoxae white. . . *pseudothetis,* new species
   Forecoxae a shade of red. 2

2. Forewing with most of wing area smoky hyaline ..... *venata*
   Forewing not smoky hyaline; completely scaled 3
3. Forewing with white apical patch.... *lenaeus*
   Forewing without white apical patch..... 4
4. Forewing with light brown apical patch (more apparent on underside of wing) *lenaeus*, variant
   Forewing without apical patch..... 5
5. Forewings with veins darker than wings *hypoleuca*
   Forewings with veins concolorus.... *redunda*

Episcepsis lenaeus (Cramer)
(Pl. II, Fig. 9)

Sphinx lenaeus Cramer, 1780: pl. 248G.
Episcepsis lenaeus, Hampson, 1898: 385.
Episcepsis lenaeus, Draudt in Seitz, 1915: 129, fig. 20b.
Episcepsis lenaeus, Kaye & Lamont, 1927: 8.

In four male specimens the white apical patch on the upperside of the forewing is greatly reduced in area and light brown rather than white. On the underside of the forewing the apical patch is also reduced in size but is more conspicuous as it is much lighter than on the upperside. The patch on the underside in two of the specimens is brownish-white rather than light brown.

Materials.—Fourteen males and two females.
Range.—Mexico to the Guianas.

Episcepsis pseudothetis, new species
(Pl. I, Figs. 1, 2)

Type Material.—All of the types were taken at Simla, Arima Valley, Trinidad. Holotype, male, Catalog No. 58101, 26-II; allotype, female, (56102) 26-XII; paratypes, males, (56103) 2-V, (56104) 14-I, (56105) 9-III, (56106) 18-IV; female, (56107) 2-I.

Disposition of Type Material.—The Department of Tropical Research, New York Zoological Society, retains paratypes 56106 and 56107. The holotypes, allotype and remaining paratypes are in the American Museum of Natural History.

Differential Diagnosis. — Episcepsis pseudothetis is superficially similar to *thetis*, but *thetis* is larger, the veins of the forewing are not contrastingly lighter than the remainder of the wing, and the apical white patch is larger as it extends further along the costal margin of the forewing. Furthermore, the hair-pencil in the hindwing is yellow in *thetis*, not almost white as in *pseudothetis*. Hampson described a species, *rhypoperas*, from Honduras that is smaller than *thetis*, has contrastingly lighter veins and a more extensive white abdominal ventrum (probably more extensive than *pseudothetis*), but the spotting about the hindhead and shoulders is orange and the hair-pencil is white but not on a pronounced projection of the inner margin of the hindwing. A species described from Venezuela, *klagesi*, has the hair-pencil on the inner margin of the hindwing yellow.

The name *pseudothetis* refers to the insect's superficial resemblance to *thetis*.

Description.—Length of forewing of male 14.5-15.5 mm., of female 16 mm. Antennae bipectinate with each pectination dilated and bristled. Palpi upturned and brown with tuft of white hair on first joint. Front and vertex of head brown. Back of head with paired crimson-colored spots and a crimson-colored spot on each side of prothorax behind eyes. Collar, tegulae and disc of thorax brown. Forecoxae white, the remainder of the legs brown. Ventrum and laterum of thorax brown.

Forewing clove brown (Ridgway, 1912, pl. 40) in fresh specimens and olive brown (*ibid.*, pl. 40) in older specimens with lighter veins, drab gray to light drab (*ibid.*, pl. 46). Apex of wing white. The white apical patch extending from the costa to vein M_1. It terminates abruptly at M_1; thus, the inner edge of the apical patch is two-sided. Underside of forewing a little darker than upperside with the apical white the same. Veins concolorous with the ground color of the wing.

Hindwing with disc semi-hyaline and margins of wing brown with a bluish cast, the latter most pronounced on the costal margin. Inner margin produced with a fold on the upper side of the wing enclosing an off-white hair-pencil in both males and females. Underside of hindwing the same as upperside but the brown color a little darker.

Abdomen with tympanic hoods conspicuous and covered with brown hair with a bluish sheen. The brown hair of the thorax is continued on the mid-dorsum of the first four abdominal segments. The remainder of the abdominal segments, including the sides of the first four segments, iridescent blue. Ventrum of abdomen with the basal three segments white, and the two subsequent segments have the anterior edge white. The caudal segments dark brown.

Episcepsis hypoleuca Hampson
(Pl. II, Fig. 10)

Heliura lamia Druce, not Butler, 1884: 74 (part).
Episcepsis hypoleuca Hampson, 1898: 388, pl. XIV, fig. 4.
Episcepsis hypoleuca, Draudt in Seitz, 1915: 130, pl. 20d.
Episcepsis inornata, Kaye & Lamont, not Walker, 1927: 8.

I do not follow Kaye & Lamont (*idem*) in synonymizing *hypoleuca* under *inornata*. The salient difference between *inornata* and *hypoleuca* is that in the latter the veins of the forewings are dark brown and in the former the veins are concolorous with the remainder of the wing. Kenedy, after examining the holotype, tells me that the wings of the holotype of *inornata* are in poor condition and that it is difficult to determine whether the veins are darker than the ground color of the wings. The photograph of the holotype shows that the wings are badly rubbed except at the proximal area of the wings. Kenedy states that the veins are concolorous with the remainder of the wings near the bases of the wings. The other difference between *inornata* and *hypoleuca* mentioned by Hampson in his key (1898: 386), namely, the dark abdominal ventrum (*inornata*) and the white abdominal ventral patches on the three basal segments (*hypoleuca*), are a sexual difference and not a difference between the species. The holotype of *inornata* is a female and the holotype of *hypoleuca* is a male. Sexual dimorphism is probably of a similar type in both species.

Hampson (1914: 243) synonymized *dodaba* Dyar under *inornata*. Forbes (1939: 142) considers *dodaba* a form of *lamia* Butler with reduced collar spots. These three forms, *inornata, dodaba* and *lamia,* differ from *hypoleuca* by having the veins of the forewing concolorous with the remainder of the wing rather than with the dark streaks on the veins characteristic of *hypoleuca.* The red spot on the anterior margin of the shoulder covers of *dodaba* and *lamia* will separate these two forms from *inornata* and *hypoleuca.*

In the Trinidad series of *hypoleuca* the males have the ventrum of the three basal segments of the abdomen white and the females have the ventrum of the abdomen black with the exception of one female specimen with a small amount of white on the basal segments.

Material.—Twenty-nine males and 18 females.

Range.—Costa Rica and Panama.

Episcepsis redunda Schaus
(Pl. II, Fig. 11)

Episcepsis redunda Schaus, 1910: 190.

Episcepsis redunda, Draudt in Seitz, 1915: 130, fig. 20c.

Episcepsis redunda, Kaye & Lamont, 1927: 8.

Material.—Only one female specimen has been collected at Simla.

Range.—Mexico to the Guianas and Peru.

Episcepsis venata Butler
(Pl. II, Fig. 12.)

Episcepsis venata Butler, 1877: 49, pl. 16, fig. 7.

Heliura aelia Schaus, 1889: 90.

Episcepsis venata, Hampson, 1889: 388.

Episcepsis venata, Draudt in Seitz, 1915: 130, fig-20d.

The smoky hyaline areas of the forewings separate this species from other Trinidad species of *Episcepsis.* The figure in Seitz is not suggestive of the species as it exists in Trinidad. The dark scaling is heavy at the tornus of the forewing in the Trinidad material as far as vein Cu_1 but may be traced to vein M_2. It extends almost halfway along the inner margin. The anterior edge of the patch lying on vein Cu_1 is directly below the discal bar. In Seitz's figure the patch is restricted to the immediate region of the anal angle. In the photograph of the holotype from "R. Jutaki, Amazons," the tornal patch is intermediate between Seitz's figure and our Trinidad material but differs from both Seitz's figure and the Trinidad material in having the area between the inner margin and vein 2d A dark-scaled to the base of the wing. The dark scaling at the apex of the forewing is reduced in Seitz's figure in comparison with the Trinidad material and the holotype. The pattern of the Trinidad material is more contrastive than either the holotype or Seitz's figure and may represent a new race.

In Kenedy's notes regarding the female holotype she mentions the presence of diffused white on the basal segments of the abdominal venter. We have one female specimen with a white venter of this nature, but the remainder of the females have a completely black abdominal ventrum. This type of variation in female abdominal ventrums is mentioned in connection with *hypoleuca.*

Material.—Six males and seven females.

Range.—Mexico to the Amazons. A new record for Trinidad.

Eriphioides Kirby

This genus, along with *Ceramidia* and *Amycles,* may be separated from other Ctenuchinae by the fact that the upper of the three posterior veins issuing from the discal cell of the hindwing is separated from the lower two connate veins by a distinct space. In other words, veins Cu_1 and Cu_2 are united and connate with vein M_3 and arise from the lower angle of the cell, while vein M_2 arises approximately a third of the way up the discocellular veins. The three genera are very closely related. Hampson distinguishes *Eriphioides* from *Ceramidia* and *Amycles* by the lateral anal abdominal tufts present in *Eriphioides.*

Eriphioides tractipennis (Butler)
(Pl. II, Fig. 13)

Eriphia tractipennis Butler, 1876: 414.

Eriphia tractipennis, Druce, 1884: 69, pl. 7, fig. 27.
Eriphioides tractipennis, Hampson, 1898: 394.
Eriphioides tractipennis, Draudt in Seitz, 1915: 132, pl. 26m.
Eriphioides tractipennis, Lamont & Callan, 1950: 197.

The abdomen of this species has a dorsal and subdorsal series of iridescent green spots. The abdominal iridescence in *Episcepsis* species is blue. The dorsum of the abdomen of the following species, *Ceramidia phemonoides,* is an immaculate iridescent cupreous green. The abdomen of *Amycles anthracina* is black-brown. Reported from Mayaro by Lamont & Callan.

Material.—One male.

Range.—Honduras to Brazil.

Ceramidia Butler

The males of this and the preceeding genus are singularized by the presence on the costal half of the hindwing of a lustrous, silky gray area.

Ceramidia phemonoides (Möschler) (Pl. II, Fig. 14)

Antichloris phemonoides Möschler, 1877, 639, pl. 8, figs. 10, 10a.
Ceramidia phemonoides, Hampson, 1898: 397.
Ceramidia phemonoides, Draudt in Seitz, 1915: 134, pl. 20i.
Ceramidia phemonoides, Kaye & Lamont, 1927: 9.

Material.—Twenty-eight males.

Range.—Venezuela, Guianas and Amazons.

Amycles Herrich-Schaeffer

For a discussion of the nomenclature of this generic name, refer to Forbes, 1939: 144.

Amycles anthracina (Walker) (Pl. II, Fig. 15)

Euchromia (*Amycles*) *anthracina* Walker, 1854: 253.
Amycles anthracina, Hampson, 1898: 398, fig. 201.
Amycles anthracina, Draudt in Seitz, 1915: 135, pl. 20i.
Amycles anthracina, Kaye & Lamont, 1927: 9.
Amycles affinis Rothschild, 1912: 153.
Amycles affinis, Hampson, 1914: 253, pl. XIII, fig. 28.
Amycles affinis, Draudt in Seitz, 1915: 135, fig. 20k.
Amycles affinis, Lamont & Callan, 1950: 197.

Hampson synonymized Felder's *adusta* under *anthracina.* Rothschild's *affinis* should also be synonymized. Walker's and Rothschild's holotypes came from Venezuela. The significant difference between *anthracina* and *affinis,* according to Rothschild's original description, is size. Rothschild gave the length of the forewing of *anthracina* as 20 mm. and of *affinis* as 14 mm. Hampson (1914: 253) gave 28-34 mm. for the wing expanse of *anthracina* and 30 mm. for the wing expanse of the holotype of *affinis.* In the photographs of the holotypes of *affinis* and *anthracina* before me, if there is any difference in the size between *affinis* and *anthracina,* the former—contrary to Rothschild's statement—is slightly larger. The specimens from Trinidad in our collection vary in length of forewing from 12 to 14 mm. The abdominal ventrum in our specimens varies slightly from dark brown to brownish-black. Kenedy states that the tarsi of the holotype of *affinis* are lighter than the rest of the legs. The color of the tarsi in our Trinidad specimens varies but is always somewhat lighter. Kenedy makes no comment regarding any difference in size between *anthracina* and *affinis,* so I conclude that any difference is negligible.

Material.—Five males and one female.

Range.—Mexico to Colombia and Brazil.

Antichloris Hübner

The males of this genus have the costal half of the hindwing clothed with lustrus, silky gray scales as in *Amycles, Eriphioides* and *Ceramidia.* However, in this genus vein Cu_2 of the hindwing is free and not united with vein Cu_1 and arises well before the end of the discal cell. One species recorded in this genus by Kaye & Lamont, *Antichloris trinitatis* Rothschild, was synonymized in Part I of this paper under *Phoenicoprocta vacillans* (Walker).

Antichloris eriphia (Fabricius) (Pl. II, Fig. 16)

Zygaena eriphia Fabricius, 1777: 276.
Sphinx alecton Stoll, 1782: pl. 382 D.
Antichloris phemonoë Hübner, 1827: pl. 9, figs. 15, 16.
Sesia melanochloros Sepp, 1848: 145, pl. 69.
Copoena scapularis Herrick-Schaeffer, 1856: fig. 260.
Chrysostola helus Herrick-Schaeffer, 1855: fig. 263.
Antichloris quartzi Klages, 1906: 548.
Antichloris eriphia, Hampson, 1898: 400.
Antichloris eriphia, Draudt in Seitz, 1915: 136, pl. 20k.
Antichloris eriphia, Kaye & Lamont, 1927: 9.

This species may be separated from the species in the other genera that have males with the costal margin of the hindwing silky by its larger size and the iridescent green streaks in the forewing.

Material.—Eleven males and one female.

Range.—Venezuela to Paraguay.

Napata Walker

The genus is made up of moths of quite varied pattern. One species, *Napata albiplaga,* might be confused with members of the preceeding four genera but has four veins from the lower part of the discal cell in the hindwing instead of the three veins characteristic of the pre-

vious genera. Vein M_2 of the hindwing is near the lower angle of the cell and not distant as in some subsequent genera.

1. Forewing with orange-yellow..... 2
   Forewing with no orange-yellow..... 3
2. Forewing orange-yellow with black and white terminal streaks at outer magin and apex of wing .....*walkeri*
   Forewing black with large triangular orange-yellow patch from base of wing to near tornus and diagonal subapical orange-yellow band .....*alternata*
3. Forewing with metallic blue spot in end of discal cell and long metallic blue spot below cell .....*albiplaga*
   Forewing without metallic blue spot or streak 4
4. Forewing with cilia at apex of wing white and hyaline streaks in and below discal cell. Wing expanse 24-27 mm.....*terminalis*
   Forewing with cilia at apex of wing concolorous with wing and subquadrangular hyaline spots in median area. Wing expanse 46 mm. .....*broadwayi*

Napata walkeri (Druce)
(Pl. II, Fig. 17)

Evius walkeri Druce, 1889: 86.
Evius walkeri, Druce, 1897: 365, pl. 73, fig. 21.
Napata walkeri, Hampson, 1898: 407.
Napata walkeri, Draudt in Seitz, 1915: 139, pl. 21c.
Napata walkeri, Kaye & Lamont, 1927: 9.

This distinctive species is very similar to another moth, *Mapeta xanthomelas* Walker, a pyralid, that also occurs in Trinidad. The black epaulet-like bars are in the spaces between the veins in *walkeri* and on the veins in *xanthomelas.*

Material.—Seventeen males and two females.

Range.—Costa Rica, Panama, Venezuela and Trinidad.

Napata alternata (Walker)
(Pl. II, Fig. 18)

Josia alternata Walker, 1864: 134.
Flavinia choana Druce, 1893: 289.
Napata alternata, Hampson, 1914: 261, pl. XIV, fig. 14.
Napata alternata, Draudt in Seitz, 1917: 208, pl. 29d.

Material.—One male collected on March 19, 1955, on *Heliotropium indicum,* during the daytime.

Range.—Venezuela, Brazil and Ecuador. A new record for Trinidad.

Napata albiplaga (Walker)
(Pl. II, Fig. 19)

Euchromia albiplaga Walker, 1854: 218.
Charidea apicalis Herrick-Schaeffer, 1854, fig. 236.
Napata albiplaga, Hampson, 1898, 409.
Napata albiplaga, Draudt in Seitz, 1915: 140, pl. 21e.

Material.—One female on May 31.

Range.—Mexico to Brazil. A new record for Trinidad.

Napata terminalis (Walker)
(Pl. II, Fig. 20)

Euchromia terminalis Walker, 1854: 231.
Napata leucotelus Butler, 1876: 409.
Napata leucotelus, Druce, 1884: 66, pl. 7, fig. 24.
Napata terminalis, Hampson, 1898: 411, pl. XIV, fig. 12.
Napata leucotelus, Hampson, 1898: 411.
Napata venezuelensis Klages, 1906: 549.
Napata teminalis, Draudt in Seitz, 1917: 141, pl. 21f.
Napata leucotelus, Draudt in Seitz, 1917: 141, pl. 21f.
Napata terminalis, Kaye & Lamont, 1927: 9.
Napata leucotelus, Kaye & Lamont, 1927: 9.

The male holotype of *terminalis* from Pernambuco, Brazil, in the British Museum (Natural History) is in good condition and the female holotype of *leucotelus* from Honduras is in poor condition. Hampson (1898: 406, 411) differentiates the two species by the relative degree of opacity of the hyaline areas in the forewings. In *terminalis* the forewing has "slight traces of hyaline patches in and below cell" or "without prominent hyaline streaks in and below cell." This is not borne out by the photograph of the male holotype of *terminalis* in which the aforementioned hyaline areas are as distinct as in the photograph of the female holotype of *leucotelus.* In our series of specimens from Trinidad the hyaline areas of the forewing vary evenly from a condition that could be called traces to a distinctly hyaline condition though even in the latter case many scales are evident under very low magnification. Our series of specimens strongly suggests that the relative distinctiveness of the hyaline areas is connected with the age of the moth. The amount of white at the apex of the forewing and tornus and inner margin of the hindwing is also variable. The only observable difference other than sex between the holotypes of *terminalis* and *leucotelus* is wing expanse size, a character of doubtful validity in this group. The holotype of *terminalis* is a male specimen and not female as Hampson states. The Trinidad material ranges in wing expanse from 24 to 27 mm. with the males slightly smaller than the females. Kenedy was unable to make any comments on the abdominal characters, due to the poor condition of the type *leucotelus.*

Material.—Sixty males and 21 females.

Range.—Mexico to Brazil.

Napata broadwayi (Schaus)

Syntomeida broadwayi Schaus, 1896: 130.

Napata broadwayi, Hampson, 1898: 413, fig. 213.
Napata broadwayi, Draudt in Seitz: 142, pl. 21g.
Napata broadwayi, Kaye & Lamont, 1927: 10.

We have not collected this species. The type in the United States National Museum is a male labelled "Trinidad." The British Museum (Natural History) has another male labelled "Trinidad" and a female from Carabaya, Peru.

Range.—Trinidad and southeast Peru.

Horama Hübner

Moths similar in facies to *Eriphioides* but with all veins present from the lower angle of the discal cell. Veins Cu_1 and Cu_2 on a long stem. No specialized scales similar to *Eriphioides*, *Ceramidia* and *Amycles* in the costal area of the hindwing of the male.

Horama oedippus (Boisduval)

Mastigocera oedippus Boisduval, 1870: 81.
Mastigocera oedippus, Druce, 1884: 49, pl. 6. fig. 19.
Horama oedippus, Hampson, 1898: 418.
Horama oedippus, Draudt in Seitz, 1915: 143, pl. 26m.
Horama oedippus, Kaye & Lamont, 1927: 10.

Kaye & Lamont (1927:10) report *oedippus* from Rock-Penal Road, Trinidad, in May. The moth has immaculate purplish-fuscus wings and may be separated from similar moths in Trinidad by the three fringes of long hair on the distal part of the tibiae. The first joint of the tarsi are fringed on each side with hair.

Range.—Mexico and Guatemala.

Cyanopepla Clemens

Large arctiid-like species, heavily scaled, often with iridescent facies and bright colors.

1. Forewing with a short crimson-colored streak below base of cell, hindwing with large crimson-colored costal patch. . . *cinctipennis*
   Forewing with long orange fascia below base of cell to beyond middle of wing, metallic green streak and crimson-colored spot beyond cell of hindwing small. . . . *submacula*

Cyanopepla cinctipennis (Walker)
(Pl. II, Fig. 21)

Charidea cinctipennis Walker, 1864: 97.
Charidea azetas Druce, 1864a: 35.
Cyanopepla cinctipennis, Hampson, 1898: 442, pl. XV, fig. 5.
Cyanopepla cinctipennis, Draudt in Seitz, 1915: 151, pl. 22g.

The black forewing with the bright red stripe at the base of the wing and the iridescent blue hindwings with a large crimson-colored patch at the outer margin of the wing distinguish this species from other ctenuchids in Trinidad.

Material.—One female specimen on March 14.

Range.—Colombia, Venezuela and Ecuador. A new record for Trinidad.

Cyanopepla submacula (Walker)
(Pl. II, Fig. 22)

Euchromia submacula Walker, 1854: 214.
Euchromia submacula, Butler, 1877: 41, pl. 13, fig. 7.
Cyanopepla submacula, Hampson, 1898: 444.
Cyanopepla submacula, Draudt in Seitz, 1915: 152, pl. 22h.
Cyanopepla submacula, Kaye & Lamont, 1927: 10.

Kaye & Lamont list this species from Port of Spain (Botanical Garden) and Morne Diable. I have specimens that F. W. Urich collected as larvae from Chaguanas on corn (*Zea mays*) and from an unknown locality on March 1, on gamelot (*Chaetochloa sulcata*). We have not collected this species in the Arima Valley.

Range.—Mexico, Guatemala, Panama and Venezuela.

Aclytia Hübner

The yellow or white spot or band on the fully-scaled forewings of the Trinidad species distinguishes these moths.

1. Fascia on forewing white . . . . . *leucaspila*, new species
   Fascia on forewing yellow. . . . . *heber*

Aclytia heber (Cramer)
(Pl. II, Fig. 23)

Sphinx heber Cramer, 1780: pl. 287 A.
Sphinx halys Stoll, 1782: pl. 357 C.
Aclytia flaviventris Möschler, 1872: 349.
Aclytia heber Hampson, 1898: 457, fig. 245.
Aclytia heber, Draudt in Seitz, 1915: 152, pl. 23f.
Aclytia heber, Kaye & Lamont, 1927: 10.

The male of these species has a yellow spot and the female an oblique yellow band on the forewing.

Material.—Three males and one female.

Range.—Central America and Cuba to Brazil.

Aclytia leucaspila, new species
(Pl. I, Figs. 3, 4)

Type Material.—Holotype, male, Catalog No. 58107, IV-5, at night, visiting *Heliotropium indicum;* paratype, male, (58108) with no date.

Disposition of Type Material.—The holotype is deposited in the American Museum of Natural History, and the Department of Tropical Research, New York Zoological Society, retains the paratype.

Differential Diagnosis. — *Aclytia leucaspila* may be distinguished from other *Aclytia* species by the white spot on the forewings. The species superficially resembles *Aclytia heber* (Cramer) which has an orange-colored spot rather than a

white spot. It is probably closely related to *Aclytia albistriga* Schaus. Schaus's original description of *Aclytia albistriga* is based on a female from Costa Rica. Forbes (Bull. Mus. Comp. Zool., LXXXV, No. 4: 146-147) had a male from Panama with the forewing banded similar to the female. *Aclytia leucaspila* may be separated from *Aclytia albistriga* by the white spot on the forewing instead of the white band.

The name, *leucaspila,* refers to the white spot on each of the forewings.

Description.—Length of forewing of male 14 mm.

Head brown. Frons edged with white at eyes and before antennae. Tongue and basal segment of palpi orange, remainder of palpi brown. Back of head with two orange-colored subdorsal spots and tegulae with orange at lateral tips.

Thorax brown. Patagia brown with an orange-white middle line. Forecoxae brown with broad white band, meso- and metathoracic coxae brown with some white scales on anterior and posterior edges. Remainder of legs brown with some white scales along posterior edge of femur.

Dorsum of abdomen dark brown with green reflections in different lights. Ventrum of abdomen white with subventral tufts of last segment brown.

Forewing dark brown, the veins lighter. The discal radial vein, the anal vein (2d A, 3d A) and the first anal fold from the base to the middle of the wing orange-gray. The remaining veins in oblique lighting cast a bluish reflection; in the same oblique lighting a blue line will form in the space below the anal vein. A suborbicular white spot at end of cell, partly in cell and bounded by veins M_1 and M_2 outside of the cell. Underside of forewing with the inner margin gray. Hindwing blackish-brown with blue reflections. A hyaline fascia below the cell and in and beyond the lower angle of the cell. Costal margin gray.

Genitalia with the left harpe narrow and right harpe much broader. The right harpe narrows abruptly from the dorsal edge at approximately two-thirds from the base. Uncus with two rounded ridges at edge in basal area which meet to form one ridge near middle. Caudal end of uncus narrow but blunt. The harpes in *Aclytia heber* are very different. The left harpe is comparatively broad with a large toothlike structure at the caudal end of the dorsal margin and the right harpe is long and needlelike, arising from an extremely broad base. The left harpe of *Aclytia gynamorpha* Hampson has a small tooth on the dorsal margin before the apex of the harpe and the right harpe stout with the end turned up sharply at right angles.

Euagra Walker

The species in this genus and the subsequent genus, *Agyrta,* are relatively large with a pattern resembling that found in the butterfly family Ithomiidae and the moth family Dysschematidae. Veins M_2 and M_3 of the hindwing are stalked in *Euagra* and approximate at origin in *Agyrta* in Trinidad species.

Euagra intercisa Butler

Euagra intercisa Butler, 1876a: 111.
Euagra intercisa, Hampson, 1898: 464, pl. XVI, fig. 8.
Euagra intercisa, Draudt in Seitz, 1915: 160, pl. 23i.
Euagra intercisa, Kaye & Lamont, 1927: 10.

Kaye & Lamont report this species from Trinidad with no locality. We have not taken it in the Arima Valley. This species lacks the apical, hyaline band in the forewing that is present in each of the three species of the next genus, *Agyrta.*

Range.—Venezuela.

Agyrta Hübner

1. Hyaline fascia below discal cell extending to base of forewing.....*dux*
   Hyaline fascia not extending to base of forewing below discal cell..... 2
2. Discal cell of forewing hyaline only in vicinity of vein Cu_2.....*micilia*
   Discal cell hyaline to near base of cell..*auxo*

Agyrta dux (Walker)
(Pl. II, Fig. 24)

Dioptis dux Walker, 1854: 327.
Agyrta aestiva Butler, 1876a: 113.
Isostola superba Druce, 1884: 115, pl. 12, fig. 5.
Agyrta phylla Druce, 1893: 282.
Agyrta dux, Hampson, 1898: 469, fig. 257.
Agyrta dux, Draudt in Seitz, 1915: 162, pl. 24a.
Agyrta dux, Kaye & Lamont, 1927: 10.

This is the largest of the three species of *Agyrta* species, the wing expanse measuring two inches or more.

Material.—Three males and two females.
Range.—Central America to Brazil.

Agyrta micilia (Cramer)
(Pl. II, Fig. 25)

Bombyx micilia Cramer, 1780: pl. 228G.
Agyrta micilia, Hampson, 1898: 470.
Agyrta micilia, Draudt in Seitz, 1915: 162, pl. 24a.
Agyrta micilia, Kaye & Lamont, 1927: 10.

Expanse of wings about one and three quarter inches.

Material.— Two males.
Range.—Panama to Brazil and Ecuador.

Agyrta auxo (Linnaeus)
(Pl. II, Fig. 26)

Sphinx auxo Linnaeus, 1767: 805.
Agyrta auxo, Hampson, 1898: 471.
Agyrta auxo, Draudt in Seitz, 1915: 162, pl. 24a.
Agyrta auxo, Kaye & Lamont, 1827: 11.

Expanse of wings about one and a half inches.

Material.—One female taken on April 29.

Range.—Venezuela to Brazil.

Delphyre Walker

Similar to *Eucereum* but veins M_3 and Cu_1 of the hindwing united.

1. Expanse of wings 24 mm. Concolorous light brown wings and abdomen.....*hebes*
   Expanse of wings 25-26 mm. Forewings gray with black-spotted facies and abdomen crimson-colored above .....*minuta*
   Expanse 30 mm. or more. Black-brown wings with distinct hyaline fascia..... 2
2. A broad milky white hyaline band beyond discal cell .....*discalis*
   A very narrow hyaline band, hardly more than streaks between the veins, beyond discal cell .....*dizona*

Delphyre hebes Walker
(Pl. II, Fig. 27)

Delphyre hebes Walker, 1854: 537.
Nodoza tristis Schaus, 1896: 150.
Delphyre hebes, Hampson, 1914: 292, pl. XVI, fig. 21.
Delphyre hebes, Draudt in Seitz, 1915: 165, pl. 29m.
Delphyre hebes, Kaye & Lamont, 1927: 11.

Material.—Ten males.

Range.—Central America and Puerto Rico to Argentina.

Delphyre minuta (Möschler)

Eucereon minutum Möschler, 1877: 651, pl. 9, fig. 19.
Delphyre minuta, Hampson, 1898: 479.
Eucereon trinita Schaus, 1901: 44.
Heliura griseipuncta Rothschild, 1912: 170.
Delphyre minuta, Hampson, 1914: 295, pl. XVI, fig. 26.
Delphyre minuta, Draudt in Seitz, 1915: 165, pl. 24d.
Eucereon trinita, Kaye & Lamont, 1927: 12.

This species is very similar to *Eucereum rosina* (p. 98), but besides the difference in venation of the hindwing, the black spots of the forewing of *rosinum* are edged with yellow. We have not collected this species in the Arima Valley. The type of the synonymized *trinita* Schaus came from Trinidad but has no specific locality.

Range.—Venezuela, Trinidad and Guianas.

Delphyre discalis (Druce)
(Pl. II, Fig. 28)

Neacerea discalis Druce, 1905: 463.
Delphyre infra-alba Rothschild, 1912: 166.
Delphyre discalis, Hampson, 1914: 301, pl. XVII, fig. 11.
Delphyre discalis, Draudt in Seitz, 1915: 165.

Material.—Thirteen males.

Range.—Described from Venezuela and recorded from French Guiana. A new record from Trinidad.

Delphyre dizona (Druce)
(Pl. II, Fig. 29)

Neacerea dizona Druce, 1898: 406.
Neacerea dizona, Hampson, 1898: 481, pl. XVI, fig. 12.
Delphyre dizona, Draudt in Seitz, 1915: 165, pl. 24d.
Delphyre dizona, Kaye & Lamont, 1927: 11.

In *dizona* both hyaline transverse bands of the forewing are narrower than in *discalis*. The inner margin of the hyaline median band in *discalis* extends to the antemedian area below the discal cell, whereas in *dizona* the inner margin of the hyaline median band is straight.

Material.—Six males.

Range.—Trinidad, British and French Guiana.

Heliura Butler

This genus is difficult to distinguish from *Eucereum* on present known generic characters. The Trinidad species, however, is a large smoky-hyaline moth with patches of dark brown scales. Laterum of abdomen iridescent blue.

Heliura suffusa (Lathy)
(Pl. II, Fig. 30)

Neacerea suffusa Lathy, 1899: 120.
Heliura suffusa, Hampson, 1914: 306, pl. XVII, fig. 19.
Heliura suffusa, Draudt in Seitz, 1915: 166 (*Delphyre*), pl. 24e.

Most species of *Heliura* have a facies resembling *Eucereum,* but *suffusa,* as its name implies, has an extremely vague pattern on the forewings. In the male almost all the hyaline area is dark and in the female the only hyaline area evident is beyond the discal cell. The semi-hyaline area in the female is much darker than the respective area in the male. The abdomen of this species is bulky and has a broad lateral band of iridescent blue scales in the male which is restricted to the basal abdominal segments in the female. The distal half of the coxae of the legs only are crimson-colored and not the whole coxae, as one might judge from the literature. Kenedy verified this feature in the holotype at the British Museum (Natural History). The species *hecale* Schaus (*picticeps* Hampson) has completely crimson-colored coxae and is presumably a sibling species. The ranges overlap in the Guianas.

Material.—Nine males and one female.

Range.—British Guiana. A new record for Trinidad.

Eucereum Hübner

Fifteen species of this genus are reported from Trinidad, the largest number of species in any one genus. The pattern of the forewings is made up of lines and spots that are in most instances difficult to describe. In the following key I have used abdominal characters and have avoided using forewing patterns wherever possible, in an attempt to simplify the key. The abdominal patterns in our Trinidad species seem to be reliable. I have included *Delphyre minuta* in the key as the facies is so similar to the typical *Eucereum* pattern. We have not collected nor have I seen specimens of *minuta* nor specimens of *Eucereum archias* and *sylvius*.

The hindwings have a remnant of vein Sc, veins R_s and M_1 approximate or very shortly stalked and vein M_2 approximate to veins M_3 and Cu_1 which are shortly stalked.

1. Abdomen immaculate bluish-black with some iridescence ..... *obscurum*
   Dorsum and laterum of abdomen with basal four segments black, then three crimson and terminal segment black..... *cinctum*
   Dorsum of abdomen immaculate crimson except for extremity..... 2
   Dorsum of abdomen with subtriangular black dorsal basal patch leaving but two or three bright-colored segments, but without mid-dorsal, black points in bright-colored segments of abdomen..... 6
   Dorsum of abdomen with black mid-dorsal points or black transverse lines on metameres of bright-colored segments of abdomen 9
2. Hindwing with only three veins from lower part of cell. Ventral surface of abdomen white ..... *Delphyre minuta*
   Hindwing with four veins present though some stalked from lower part of cell. Ventrum of abdomen yellowish or pinkish..... 3
3. Hindwing black-brown with small semi-hyaline area below lower part of cell..... 4
   Hindwing hyaline white with blackish-brown restricted to margin of wing..... 5
4. Ventrum of abdomen pink..... *mitigata*
   Ventrum of abdomen yellow.. *archias* (male)
5. Ventrum of abdomen pink..... *rosina*
   Ventrum of abdomen yellow. *archias* (female)
6. Upper and lower sides of forewings without semi-hyaline white spots.... *pseudoarchias*
   Upper and lower sides of forewings with some hyaline white spots..... 7
7. Underside of abdomen dark brown *hyalinum* (some)
   Underside of abdomen pink..... 8
8. Ground color of hindwing light brown and hyaline ..... *latifascia*
   Ground color of hindwing dark brown. *sylvius*
9. Abdomen with black triangular or quadrate patch leaving only two or three bright-colored dorsal segments..... 10
   Abdomen with the larger part of abdomen bright-colored 12
10. Abdomen orange-yellow. Hindwing white hyaline with narrow, well defined terminal dark border ..... *setosum*
    Abdomen carmine. Hindwing with wide terminal border 11
11. Subdorsum of abdomen carmine. Basal patch quadrate. Black points on bright-colored segments. Underside pink... *obliquifascia*
    Subdorsum of abdomen concolorous with dark basal patch. Usually transverse metamere streaks on bright-colored segments. Underside of abdomen dark brown *hyalinum* (most)
12. Underside of forewing without semi- or milky hyaline patches ..... *dentatum*
    Underside of forewing with some semi- or milky hyaline patches..... 13
13. Ground color of forewing white *dorsipunctum*
    Ground color of forewing brown..... 14
14. Whole of dorsum of basal two abdominal segments brown ..... *aeolum*
    Only the mid-dorsum of basal abdominal segments brown ..... *maia*

Eucereum archias (Stoll)

Sphinx archias Stoll, 1790: pl. 14, figs. 6-10.
Eucereon archias, Hampson, 1898: 485, fig. 269.
Eucereon archias, Draudt in Seitz, 1915: 170, pl. 24g.
Eucereon archias, Kaye & Lamont, 1927: 11.

Kaye & Lamont record this species from Palmiste and the larvae on orange. We have not taken it at Simla. It is the type species of the genus but is distinctive from other species in the genus in having long lateral pencils of hair from the basal abdominal segment in the male.

Eucereum cinctum Schaus
(Pl. III, Fig. 1)

Eucereon cinctum, Schaus, 1896: 134.
Eucereon cinctum Hampson, *nec* Schaus, 1898: 486, fig. 271.
Eucereon cincta, Hampson, 1914: 317, 234, pl. XVIII, fig. 19.
Eucereum cinctum, Draudt in Seitz, 1915: 171.
Eucereon cinctum, Kaye & Lamont, 1927: 11.

The holotype of this species was described from Aroa, Venezuela, not Trinidad as reported in Kaye & Lamont and Draudt.

Material.—Six males and four females.
Range.—Venezuela and Trinidad.

Eucereum obscurum (Möschler)
(Pl. III, Fig. 2)

Aclytia obscura Möschler, 1872: 348.
Epanycles stellifera Butler, 1877: 48, pl. 16, fig. 10.

Eucereon obscurum, Hampson, 1898: 490.
Eucereum obscurum, Draudt in Seitz, 1915: 171, pl. 24g.

The facies of this species is not of the type associated with *Eucereum.* However, the bluish-black abdomen, combined with black wings overlaid with a complex pattern of gray or white scales, is distinctive.

Material.—Forty males and eight females.

Range.—Mexico to the Amazons. A new record for Trinidad.

Eucereum rosina (Walker)
(Pl. III, Fig. 3)

Euchromia rosina Walker, 1854: 270.
Carales imprimata Walker, 1864: 305.
Eucereum rhodophila Druce, *nec* Walker, 1884: 86 (in part).
Eucereon rosinum, Hampson, 1898: 492, pl. XVI, fig. 18.
Eucereum rosina, Draudt in Seitz, 1915: 172, pl. 24i.
Eucereon rosinum, Kaye & Lamont, 1927: 11.

The small size and the immaculate carmine-colored abdomen are distinctive.

Material.—Thirty-nine males and two females.

Range.—Mexico to southern Brazil.

Eucereum mitigata Walker
(Pl. III, Figs. 4, 5)

Eucerea mitigata Walker, 1856: 1639.
Eucereon punctatum Hampson, not Guerin-Meneville, 1898: 494.
Eucereon punctatum, Hampson, not Guerin-Meneville, 1914: 319.
Eucereum punctatum, Draudt in Seitz, not Guerin-Meneville, 1915: 173, pl. 24k.
Eucereon punctata, Kaye & Lamont, not Guerin-Meneville, 1927: 12.

This species is confused in Hampson. The description under *punctatum* (1898: 494) is basically of *mitigata* Walker, not of *punctatum* Guerin-Meneville. The photograph taken by Kenedy of the holotype of *Chalonia punctata* in the Oxford Museum collection is of a *Eucereum* with a facies resembling *marcata;* the background area is large and the spotting small and irregularly linear rather than round and orbicular. The label on the photograph of the holotype of *punctata* has a locality which I decipher as Campeche, which is most likely to be the Yucatan Peninsula. There are three other species that are probably associated with *mitigata. Eucereum reticulata* Butler from the Amazons is either a synonym or a sibling species, judging from the photograph of the type. The only difference I can observe in the photograph is that the spots on the forewing of *reticulata* are larger, particularly those spots on each side of the anal vein in the median part of the forewing. *Eucereum ruficollis* Lathy is very similar to our Trinidad females. A photograph of the holotype of *ruficollis* shows denser—or in other words larger—spots making up the median transverse band in the forewing. *Eucereum zamorae* from Guatemala is also very similar to our Trinidad females if one is to judge on the basis of Hampson's figures (1914: pl. XVIII, fig. 10). It differs in having a line from the terminal spot at the outer margin near the tornus joining the post-median band in the vicinity of vein Cu_2.

The figure in Seitz (24k) of *punctatum* is a good match for our Trinidad male *mitigata.* The females have a very white background and the spots are more distant.

Material.—Sixteen males and three females.

Range.—Brazil (Para) and Trinidad.

Eucereum hyalinum Kaye
(Pl. III, Fig. 6)

Eucereon hyalinum Kaye, 1901: 119, pl. V, fig. 11.
Eucereon hyalina, Hampson, 1914: 323.
Eucereum hyalinum, Draudt in Seitz, 1915: 173, pl. 24k.
Eucereon hyalinum, Kaye & Lamont, 1927: 12.

This species was described from Arima. Besides the abdominal differences mentioned in the key, this species may be separated from the very similar *latifascia* by the following characters: the thorax of *hyalinum* is dark brown; of *latifascia,* gray-brown with a fine black mid-dorsal line. The patagia of *hyalinum* is dark brown with a light brown line; of *latifascia,* light brown with a dark line. The round spot on the mid-dorsum of the metathorax of *hyalinum* is light gray and orbicular; of *latifascia,* largely dark brown with two gray streaks on each side of the caudal edges. The pattern of the forewings of *hyalinum* has the dark spots more amalgamated than *latifascia,* in which each spot is quite nicely margined by the lighter ground color. The gray-white spot on the outer margin of *hyalinum* has two black streaks within it, whereas in *latifascia* the white patch is displaced inwards from the outer margin and the two streaks lie outside of it. In addition to the abdominal characters mentioned in the key the ventrum of *hyalinum* is brown and the ventrum of *latifascia* is pink.

Material.—Four males and one female.

Range.—Trinidad and British Guiana.

Eucereum dentatum Schaus
(Pl. III, Fig. 7)

Eucereon dentatum Schaus, 1894: 229.
Eucereon dentata, Hampson, 1914: 329, pl. XVIII, fig. 26.
Eucereum dentatum, Draudt in Seitz, 1915: 174, pl. 24k.
Eucereon dentatum, Forbes, 1939: 157.

Schaus does not give the sex of the holotype.

Kenedy, who inspected the type in the U. S. National Museum, notes that it is a female. Hampson's figure (1914) of this species shows the basal half of the forewing a much darker color than our single male from Trinidad. Our specimen agrees with the figure in Seitz (1915) in which the color of the basal half of the forewing is but slightly darker than the remainder of the wing.

Material.—One female on March 26.

Range.—Mexico to Ecuador and Venezuela. A new record for Trinidad.

Eucereum dorsipunctum (Hampson)
(Pl. III, Fig. 8)

Eucereon dorsipuncta Hampson, 1905: 430.
Eucereon dorsipuncta, Hampson, 1914: 320, pl. XVIII, fig. 12.
Eucereum dorsipunctum, Draudt in Seitz, 1915: 174, pl. 30e.
Eucereon dorsipunctum, Lamont & Callan, 1950: 197.

Material.—One female on April 5, on *Heliotropium indicum.*

Range.—Venezuela, Bolivia, Paraguay to southern Brazil.

Eucereum sylvius (Stoll)

Sphinx sylvius Stoll, 1790: pl. 14, figs. 1-5.
Eucereon sylvius, Hampson, 1898: 497.
Eucereum sylvius, Draudt in Seitz, 1915: 175, pl. 25a.
Eucereon sylvius, Kaye & Lamont, 1927: 12.

We have not captured this species at Simla. The two records given by Kaye & Lamont are in the west central part of Trinidad.

Range.—Reported from Mexico to the Amazons.

Eucereum pseudoarchias Hampson
(Pl. III, Fig. 9)

Eucereon pseudoarchias Hampson, 1898: 497, fig. 272.
Eucereum pseudoarchias, Draudt in Seitz, 1915: 175, pl. 25a (f. *aurantiaca*).
Eucereon pseudoarchias, Kaye & Lamont, 1927: 12.

The wing facies and abdominal pattern are similar in our single female to the males.

Material.—Ten males and one female.

Range.—Mexico to the Amazons.

Eucereum aeolum Hampson
(Pl. III, Fig. 10)

Eucereon aeolum Hampson, 1898: 498, pl. XVI, fig. 16.
Eucereum aeolum, Draudt in Seitz, 1915: 175, pl. 25a.
Eucereon aeolum, Kaye & Lamont, 1927: 12.

Kenedy states that the holotype is a male. Kaye & Lamont record the species from Trinidad but with no locality. It is common at Simla.

Material.—Thirteen males and 11 females.

Range.—Mexico to Peru, Venezuela and Trinidad.

Eucereum latifascia Walker
(Pl. III, Fig. 11)

Eucerea latifascia Walker, 1856: 1639.
Eucereon latifascia, Hampson, 1898: 498, pl. XVI, fig. 14.
Eucereum latifascia, Draudt in Seitz, 1915: 176, pl. 25a.
Eucereon latifascia, Kaye & Lamont, 1927: 12.

It is with reluctance that I employ the above name. As I use it, it agrees with the collection in the British Museum (Natural History), which in turn is probably based on a specimen labelled "compared with type at Oxford, 1880." Kenedy thinks our specimens are essentially the same as this one. One specimen from Arima is in the Kaye collection as *latifascia* and is similar to our series.

The following paragraph refers only to the holotype of *latifascia* Walker and not to *latifascia* of authors or my own use of the name in this paper. The following comments are based on a photograph of the holotype in the Oxford Museum.

The photograph of the holotype of *latifascia* is that of a form very similar to *hyalinum.* The facies of the forewing of *latifascia* (holotype) is similar to *hyalinum* in the disposition of the dark spots and the light patch on the outer margin. The mid-dorsal light spot on the caudal part of the meta-thorax appears to be similar. However, the dorsal abdominal pattern is different. The *latifascia* (holotype) of the Oxford Museum has the basal abdominal segments dark with the subsequent abdominal segment figured with a mid-dorsal triangular dark patch with the apex of the triangle at the dark basal abdominal segments. The three subsequent bright segments appear to be immaculate, whereas in *hyalinum* there are dark transverse lines on the metameres of the bright-colored segments. The ventrum of the abdomen of both *latifascia* (holotype) and *hyalinum* is dark brown. The facies of both the fore- and hindwing of *latifascia* (holotype) appear to be very similar to *varium* Walker. The abdomen of *varium* is similar except that the dark patch on the segment subsequent to the dark basal segments is quadrate in *varium* and triangular in *latifascia* (holotype). My comments on *varium* are based on the figure of *varium* in Seitz (1915, pl. 25e).

Although in using the name *latifascia* for the Trinidad material I am apparently perpetuating an error, I consider that naming a new species in this confusing complex of *Eucereum* species would create greater confusion. The elucidation

of this section must await the proper examination of the types and a good series of specimens. Our Trinidad *latifascia* closely resembles the figure in Seitz (1915, pl. 25a). However, in the forewing the light spot near the outer margin of the wing is larger and the bright-colored segments of the abdomen do not have mid-dorsal spots. Hampson's figure of *latifascia* (1898: 498, pl. XVI, fig. 14) does not resemble our specimens, the holotype at the Oxford Museum or the specimens under the name of *latifascia* in the British Museum (Natural History).

The four male genitalia that I have studied in the Trinidad material are variable.

Material.—Thirteen males and one female.

Range.—Draudt in Seitz reports his form from Mexico to Peru and Brazil.

Eucereum obliquifascia Rothschild
(Pl. III, Fig. 12)

Eucereon obliquifascia Rothschild, 1912: 175.
Eucereon obliquifascia, Hampson, 1914: 329, pl. XVIII, fig. 27.
Eucereum obliquifascia, Draudt in Seitz, 1915: 176, pl. 30g.
Eucereon obliquifascia, Kaye & Lamont, 1927: 13.

Reported only from Trinidad. The type is from Port of Spain, and the British Museum (Natural History) has one other specimen from Caparo, Trinidad. The figures in Hampson and Seitz are unrecognizable. The spots on the forewing are larger and more irregular. An oblique band made up of spots crosses the forewing from the costa through the discal cell to the tornus. A light brownish-gray spot within the cell and four beyond the cell are not shown in the figures.

Material.—Four males.

Range.—Trinidad.

Eucereum maia Druce
(Pl. III, Fig. 13)

Eucereon maia Druce, 1884: 86, pl. 9, fig. 13.
Eucereon maia, Hampson, 1898: 499.
Eucereum maja, Draudt in Seitz, 1915: 176, pl. 25b.
Eucereon maia, Kaye & Lamont, 1927: 13.

The commonest species of *Eucereum* at Simla. The forewings have a very noctuid-like pattern.

Material.—Seventy males and eight females.

Range.—Central America to the Amazons.

Eucereum setosum (Sepp)
(Pl. III, Fig. 14)

Phalaena setosa Sepp, 1830: pl. 9.
Eucereon setosum, Hampson, 1898: 507.
Eucereum setosum, Draudt in Seitz, 1915: 179, pl. 25f.
Eucereon mara Kaye, 1914: 115.
Eucereum mara, Draudt in Seitz, 1915: 179.

Material.—One male.

Range.—Mexico to Brazil. A new record for Trinidad.

Correbia Herrich-Schaeffer

This and the following genus resemble lycid beetles. In the present genus, veins M_2 and M_3 of the forewing are on a long stem. Only one species has been found in Trinidad.

Correbia lycoides (Walker)
(Pl. II, Fig. 31)

Euchromia lycoides Walker, 1854: 256.
Euchromia lycoides, Butler, 1877: 47, pl. 8, fig. 10.
Correbia ceramboides, Herrich-Schaeffer, 1855: fig. 265.
Correbia lycoides, Hampson, 1898: 515, fig. 273.
Correbia lycoides, Draudt in Seitz, 1915: 185, pl. 25k.
Correbia lycoides, Kaye & Lamont, 1927: 13.

Material.—Twenty-nine males and 22 females.

Range.—Mexico to Brazil.

Correbidia Hampson

This genus differs from the preceding in having veins M_2 and M_3 of the forewing approximate or united for a very short distance. Forbes (1939: 160) suspects that many of the species are really forms of a single species. He places *striata, elegans, costinota* and *germana* as Central American forms, *bicolor, apicalis* and *terminalis* as Antillean, and *calopteridia, assimilis* and *cimicoides* as South American.

1. Forewing blackish and median band light colored 2
   Forewing yellowish and median band black 3
2. Median band of forewing whitish....*notata*
   Median band of forewing buff.....*assimilis*
3. Base of forewing black, ventrum of terminal abdominal segments yellow...*calopteridia*
   Base of forewing yellow, ventrum of terminal abdominal segments black.....*terminalis*

Correbidia notata (Butler)

Pionia notata Butler, 1878: 45.
Correbidia notata, Hampson, 1898: 518, pl. XVII, fig. 3.
Correbidia notata, Draudt in Seitz, 1915: 187, pl. 26a.
Correbidia notata, Kaye & Lamont, 1927: 13.

Kaye & Lamont list this species from Trinidad with no further data. We have not collected it.

Range.—Trinidad and Amazons.

Correbidia assimilis (Rothschild)
(Pl. II, Fig. 32)

Correbia assimilis Rothschild, 1912: 182.
Correbia similis Rothschild, 1912: 182.
Correbidia assimilis, Hampson, 1914: 363, pl. XX, fig. 31.
Correbidia similis, Hampson, 1914: 364, pl. XX, fig. 32.

Correbidia assimilis, Draudt in Seitz, 1915: 187, pl. 31g.
Correbidia similis, Draudt in Seitz, 1915: 187, pl. 31g.
Correbidia similis, Kaye & Lamont, 1927: 13.

The Trinidad material varies from *similis* to *assimilis.* The only difference between *similis* and *assimilis* as described is that the yellow patch at the base of the forewing is wider in *similis* than in *assimilis.* This widening of the yellow at the base of the wing is done at the expense of the width of the dark band crossing the cell. In the Kenedy photographs of the types before me, the dark band also extends towards the base of the wing just below the cell in *assimilis* but only along the inner margin in *similis.* As mentioned above, however, our material grades from the one form to the other in such an even way as to make division of the forms impossible. In a few instances a single specimen will show variation between the wings of one side and the other. Hampson's figures, while suggestive of the species, are unreliable. First, the buff band near the black apical patch of the forewing is narrower in *similis* than in *assimilis,* the reverse of Rothschild's description. In point of fact, judging from the photographs of the types, the buff band is of equal width in both forms. It is the median black band that varies in width. In addition, the inner margin of the black apical patch does not arch towards the base of the wing as shown in the figure of *similis,* but on the contrary, indents towards the outer margin along vein Cu_1 in the form of a small notch as is shown in Hampson's figure of *assimilis.* The color of the light bands is the same in both forms and not lighter in *similis* as Hampson's figures would lead one to believe. In the photographs of the types the inner margin is narrowly black from the black median band to the base of the wing in *similis* and broad in *assimilis.* The character is not shown in the figures. Seitz's figures are even more unreliable.

I have selected the name *assimilis* on the basis of paragraph priority.

Material.—One hundred and twenty-five males and 24 females.

Range.—Rothschild described *similis* from material coming from Venezuela (holotype), Peru and Trinidad, and *assimilis* from Venezuela (holotype), British Guiana, Surinam and lower and upper Amazons.

Correbidia calopteridia (Butler)

Pionia calopteridia Butler, 1878: 381.
Correbidia calopteridia, Hampson, 1898: 518, pl. XVII, fig. 22.
Correbidia calopteridia, Draudt in Seitz, 1915: 187, pl. 26a.
Correbidia calopteridia, Kaye & Lamont, 1927: 13.

We have not collected this species, but Kaye & Lamont report a specimen from Port of Spain.

Range.—Trinidad, Guianas, northern Brazil and Peru.

Correbidia terminalis (Walker)

Pionia terminalis Walker, 1856: 1633.
Charidea cimicoides Herrich-Schaeffer, 1866: 116.
Correbidia terminalis, Hampson, 1898: 519, fig. 274.
Correbidia terminalis, Draudt in Seitz, 1915: 187.
Correbidia terminalis, Kaye & Lamont, 1927: 13.

Collected at Palmiste by Kaye & Lamont but we have not collected it in the Arima Valley.

Range.—Difficult to determine because of misidentifications, but Forbes (1939: 161) gives it as the Greater Antilles.

Ctenucha Kirby

A varied genus of mostly slim-bodied and broad-winged species, some of which have a facies resembling arctiids and, in the case of the Trinidad species, a geometrid. The genus ranges from Canada to Paraguay.

Ctenucha andrei Rothschild
(Pl. II, Fig. 33)

Ctenucha andrei Rothschild, 1912: 184.
Ctenucha andrei, Hampson, 1914: 374, pl. XXI, fig. 18.
Ctenucha andrei, Draudt in Seitz, 1915: 190, pl. 31k.
Ctenucha andrei, Kaye & Lamont, 1927: 13.

The facies of this moth resemble a geometrid rather than a ctenuchid. It has very broad bluish-black wings with a white transverse bar. The holotype of this species came from Ariapite Valley, Trinidad.

Material.—Thirteen males and two females.

Range.—Trinidad.

References

Beebe, W.
1952. Introduction to the ecology of the Arima Valley, Trinidad, B.W.I. Zoologica, 37: 157-184.

1953. A contribution to the life history of the euchromid moth, *Aethria carnicauda* Butler. Zoologica, 38: 155-160.

1955. Two little-known selective insect attractants. Zoologica, 40: 27-32.

Boisduval, J. A.
1870. Considerations sur les Lépidoptères envoyés du Guatemala à M. de l'Orza. Paris: 1-100.

Butler, A. G.
1876. Notes on the Lepidoptera of the family Zygaenidae, with descriptions of new gen-

era and species. Linn. Soc. Zool., 12: 342-433.

1876a. On the Lepidoptera referred by Walker to the genus *Dioptis* of Hubner. Cist. Ent., 2: 107-121.

1877. Illustrations of typical specimens of Lepidoptera Heterocera in the collection of the British Museum. Pt. I: xiii + 62.

1878. On the Lepidoptera of the Amazons, collected by Dr. James W. H. Trail during the years 1873 to 1875. Trans. Ent. Soc. Lond., 1878: 39-84.

Cramer, P.

1775-1796. Papillons exotiques des trois parties du monde. Amsterdam. Suppl. by Stoll.

Dognin, P.

1911. Hétérocères nouveaux de l'Amérique du Sud. Rennes, 2: 3-56.

1923. Hétérocères nouveaux de l'Amérique du Sud. Rennes, 24: 1-34.

Draudt, M., in A. Seitz

1915-1917. Macrolepidoptera of the World. Syntomidae. Stuttgart, VI: 33-230.

Druce, H.

1884. Biologia Centrali-Americana. London. Insecta, Lepidoptera Heterocera, 1: xxxi + 490.

1889. Descriptions of new species of Lepidoptera, chiefly from Central America. Ann. Mag. Nat. Hist., (6) 4: 77-94.

1893. Descriptions of new species of Lepidoptera Heterocera from Central and South America. Proc. Zool. Soc. Lond., 1893: 280-310.

1896. Biologia Centrali-Americana. London. Insecta, Lepidoptera Heterocera, 2: 1-622.

1896a. Descriptions of some new genera and species of Heterocera from Central and tropical South America. Ann. Mag. Nat. Hist., (6) 18: 28-42.

1897. Descriptions of some new species of Heterocera from tropical America. Ann. Mag. Nat. Hist., (6) 20: 299-305.

1898. Descriptions of some new species of Syntomidae chiefly in the Oxford Museum. Ann. Mag. Nat. Hist., (7) 1: 401-408.

1905. Descriptions of some new species of Syntomidae and Arctiidae from tropical South America. Ann. Mag. Nat. Hist., (7) 15: 460-467.

1906. Descriptions of some new species of Heterocera from tropical South America. Ann. Mag. Nat. Hist., (7) 18: 77-94.

Drury, D.

1770-1775. Illustrations of Exotic Entomology. London.

Dyar, H. G.

1899. New species of Syntomidae. Journ. N. Y. Ent. Soc., 7: 174-176.

Fabricius, J. C.

1777. Genera insectorum, cum mantissa speciarum nuper desectarum. Chilonii: 209-310.

1781. Species insectorum. Hamburgii et Chilonii, 2: 1-382.

Felder, C.

1864-1867. Reise der osterreichischen Fregatte Novara um die Erde., Zoologischer Theil, 2 Wien: vi + 548.

Fleming, H.

1957. The Ctenuchidae (Moths) of Trinidad, B.W.I. Part I. Euchromiinae. Zoologica, 42: 105-130.

Forbes, W. T. M.

1939. Lepidoptera of Barro Colorado Island, Panama. Bull. Mus. Comp. Zool., 85, No. 4: vii + 97-332.

Hampson, G. F.

1898. Catalogue of the Lepidoptera Phalaenae in the British Museum. London. I: xxi + 559.

1905. Descriptions of new genera of Syntomidae, Arctiadae, Agaristidae and Noctuidae. Ann. Mag. Nat. Hist., (7) 15: 425-453.

1909. Descriptions of new genera and species of Syntomidae, Arctiadae, Agaristidae, and Noctuidae. Ann. Mag. Nat. Hist., (8) 4: 344-388.

1914. Catalogue of the Lepidoptera Phalaenae in the British Museum, London. Supplement 1: xxviii + 858.

Herrich-Schaeffer, G. A. W.

1850-1858. Sammlung neuer oder wenig bekannter aussereuropäischer Schmetterlings. Regensburg: 1-84.

1866. Schmetterlinge aus Cuba. Corresp. Blatt. Zool. —min. Ver. Regenst., 20: 113-120.

Hübner, J.

1827. Zutrage zur Sammlung exotischer Schmetterlinge. Augsburg: 1-48.

Jones, E. D.

1914. New species of Lepidoptera Heterocera from southeast Brazil. Trans. Ent. Soc. Lond., 1914: 1-12.

Kaye, W. J.

1901. A preliminary catalogue of the Lepidoptera Heterocera of Trinidad. Trans. Ent. Soc. Lond., 1901: 115-158.

1911. New species of Syntomidae from British Guiana and south Brazil. Entomologist, 44: 142-146.

1914. New species of Syntomidae from Venezuela. Ent. Rec. and Journ. Variation, 26: 115-116.

1924. New species and subspecies of Trinidad Rhopalocera and Heterocera. Trans. Ent. Soc. Lond., 1924: 413-428.

KAYE, W. J., & N. LAMONT
1927. A catalogue of the Trinidad Lepidoptera Heterocera (moths). Mem. Dept. Agric., Trinidad and Tobago, No. 3: iv + 144.

KLAGES, E. A.
1906. On the syntomid moths of southern Venezuela collected in 1898-1900. Proc. U. S. Nat. Mus., 29: 531-552.

LAMONT, N., & E. M. CALLAN
1950. Moths new to Trinidad, B.W.I. Zoologica, 35: 197-207.

LATHY, P. I.
1899. Descriptions of new species of Syntomidae in the collection of Mr. H. J. Adams, F. E. S. Entomologist, 32: 116-121.

LINNAEUS, C. V.
1758. Systema naturae. Stockholm, 10th ed.: 1-824.
1767. *Ibid.* Stockholm, 12th ed.: 1, pt. 2: 533-1325.

MÖSCHLER, H. B.
1872. Neue exotische Schmetterlinge. Stett. Ent. Zeit., 33: 336-362.
1877. Beitrage zur Schmetterlings fauna von Surinam. Verh. Zool.-bot. Ges. Wien., 27: 629-700.
1883. Beitrage zur Schmetterlings fauna von Surinam V. (Supplement). Verh. Zool.-bot. Ges. Wien., 32: 303-362.

ORFILA, R. N.
1935. Lepidoptera Neotropica. 1, 2. Rev. Soc. Ent. Argent., 7: 177-182, 225-226.

PALLAS, P. S.
1772. Spicilegia Zoologica, Berolini. (Berlin) 1-13: 1-86.

PERTY, J. A. M.
1830. Delectus Animalium Articulaterum quae in Itinereper Brazilian Annio 1817-1820. Monachii: iii + 223.

RIDGWAY, R.
1912. Color standards and color nomenclature. Washington, D. C.: iii + 43.

ROTHSCHILD, W.
1911. New Syntomidae in the Tring Museum. Nov. Zool., 18: 24-45.
1912. New Syntomidae. Nov. Zool., 19: 151-186.
1913. Some unfigured Syntomidae. Nov. Zool., 20, pl. 13-14.

SCHAUS, W.
1889. Descriptions of new species of Mexican Heterocera. Ent. Amer., V: 87-90.
1892. Descriptions of new species of Lepidoptera Heterocera from Brazil, Mexico, and Peru. Proc. Zool. Soc. Lond., 1892: 272-291.
1894. On new species from tropical America. Proc. Zool. Soc. Lond., 1894: 225-243.
1896. New species of American Heterocera. Journ. N. Y. Ent. Soc., 4: 130-145.
1901. Heterocera from tropical America. Journ. N. Y. Ent. Soc., 9: 40-48.
1905. Descriptions of new South American moths. Proc. U. S. Nat. Mus., 29: 179-345.
1910. Descriptions of new Heterocera from Costa Rica. Ann. Mag. Nat. Hist., (8) 6: 189-211.
1920. New species of Lepidoptera in the U. S. Nat. Mus. Proc. U. S. Nat. Mus., 57: 107-152.

SEPP, C.
1830. Surinaamsche Vlinders, naar het, leven geteekned. Amsterdam. (1828-1854), I, II, III.
1848. Surinaamsche Vlinders, naar het, leven geteekned. Amsterdam. (1828-1854), I, II, III.

STOLL, P., in P. CRAMER
1775-1796. Papillons exotiques des trois parties du monde. Amsterdam. Suppl. by Stoll.

STRAND, E.
1915. Uber einige exotische (exklus. Asiatische) Syntomididen des Deutschen Entomologischen Museums. Dtsch. Ent. Ztschr., 1915: 19-35.
1917. Neue und wenig bekannte Nebenformen von Syntomididen. Arch. Natg. Berlin Abt., A 82, Haft 2 (1916): 79-86.

TRAVASSOS FILHO, L.
1957. Contribuicao ao conhecimento dos Ctenuchidae (Lep.) VII-Genero *Dinia* Walker, 1854. Arq. Zool., 10: 185-207.

WALKER, F.
1854. List of the specimens of lepidopterous insects in the collection of the British Museum. London. Lepidoptera Heterocera, I: 1-278.
1856. *Ibid,* VII: 1509-1808.
1864. *Ibid,* XXXI: (Suppl.) 1-321.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. *Episcepsis pseudothetis,* new species. Holotype, Catalog No. 58101 (male).
Fig. 2. *Episcepsis pseudothetis,* male genitalia.
Fig. 3. *Aclytia leucaspila,* new species. Holotype, Catalog No. 58107 (male).
Fig. 4. *Aclytia leucaspila,* male genitalia.

Plate II

Fig. 1. *Dinia aeagrus.*
Fig. 2. *Trichura fumida.*
Fig. 3. *Trichura coarctata.*
Fig. 4. *Aethria carnicauda.*
Fig. 5. *Aethria aner.*
Fig. 6. *Urolasia brodea.*
Fig. 7. *Chrysostola fulvisphex.*
Fig. 8. *Cercopimorpha dolens.*
Fig. 9. *Episcepsis lenaeus.*
Fig. 10. *Episcepsis hypoleuca.*
Fig. 11. *Episcepsis redunda.*
Fig. 12. *Episcepsis venata.*
Fig. 13. *Eriphioides tractipennis.*
Fig. 14. *Ceramidia phemonoides.*
Fig. 15. *Amycles anthracina.*
Fig. 16. *Antichloris eriphia.*
Fig. 17. *Napata walkeri.*
Fig. 18. *Napata alternata.*
Fig. 19. *Napata albiplaga.*
Fig. 20. *Napata terminalis.*
Fig. 21. *Cyanopepla cinctipennis.*
Fig. 22. *Cyanopepla submacula.*
Fig. 23. *Aclytia heber.*
Fig. 24. *Agyrta dux.*
Fig. 25. *Agyrta micilia.*
Fig. 26. *Agyrta auxo.*
Fig. 27. *Delphyre hebes.*
Fig. 28. *Delphyre discalis.*
Fig. 29. *Delphyre dizona.*
Fig. 30. *Heliura suffusa.*
Fig. 31. *Correbia lycoides.*
Fig. 32. *Correbidia assimilis.*
Fig. 33. *Ctenucha andrei.*

Plate III

Fig. 1. *Eucereum cinctum.*
Fig. 2. *Eucereum obscurum.*
Fig. 3. *Eucereum rosina.*
Fig. 4. *Eucereum mitigata.*
Fig. 5. *Eucereum mitigata.*
Fig. 6. *Eucereum hyalinum.*
Fig. 7. *Eucereum dentatum.*
Fig. 8. *Eucereum dorsipunctum.*
Fig. 9. *Eucereum pseudoarchias.*
Fig. 10. *Eucereum aeolum.*
Fig. 11. *Eucereum latifascia.*
Fig. 12. *Eucereum obliquifascia.*
Fig. 13. *Eucereum maia.*
Fig. 14. *Eucereum setosum.*

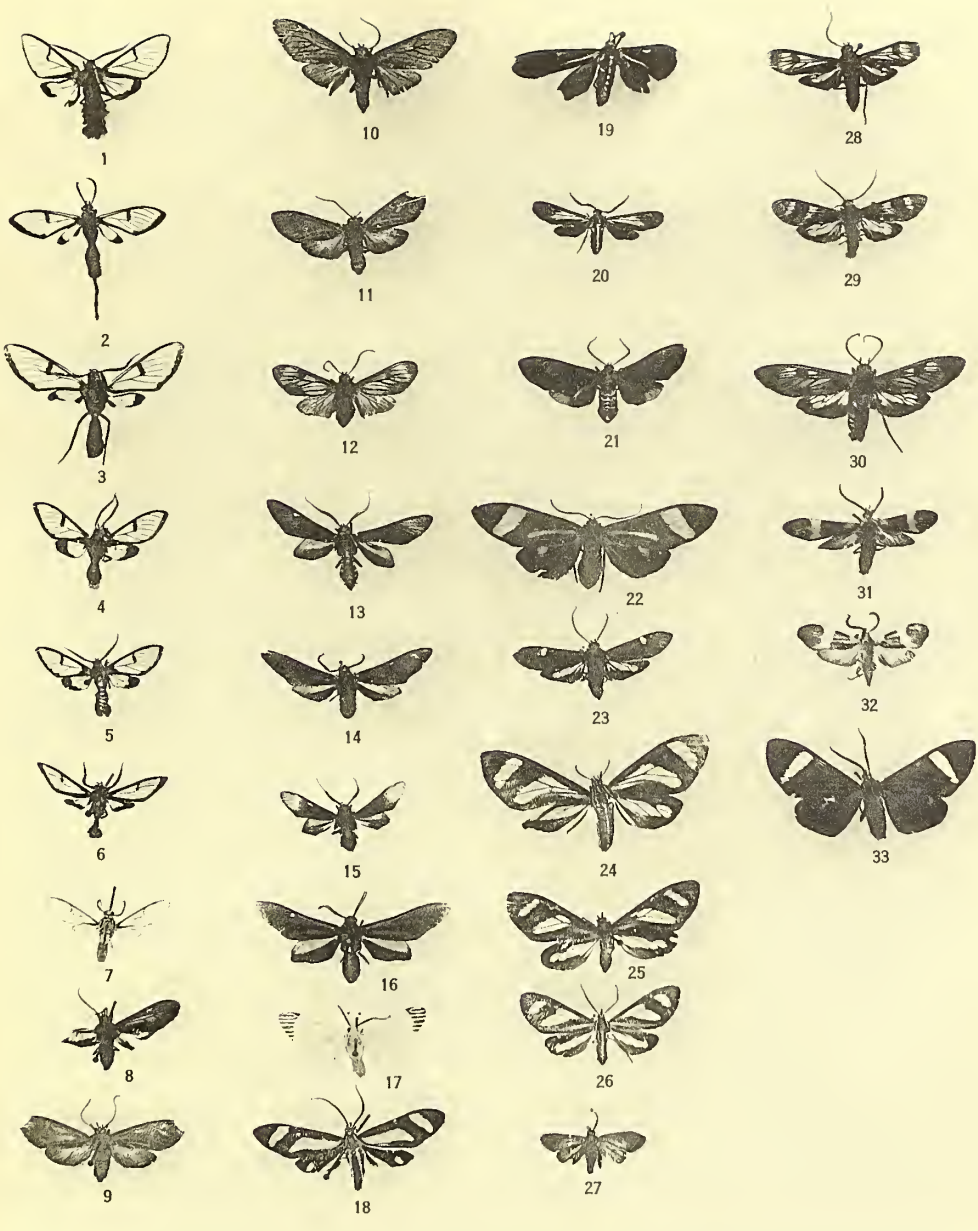
FIG. 1

FIG. 2

FIG. 3

FIG. 4

THE CTENUCHIDAE (MOTHS) OF TRINIDAD, B.W.I.
PART II. CTENUCHINAE.



THE CTENUCHIDAE (MOTHS) OF TRINIDAD, B.W.I.
PART II. CTENUCHINAE.

1

6

10

2

7

11

3

8

12

4

9

13

5

14

THE CTENUCHIDAE (MOTHS) OF TRINIDAD, B.W.I.
PART II. CTENUCHINAE.

7

Eastern Pacific Expeditions of the New York Zoological Society. XLIV. Non-intertidal Brachygnathous Crabs from the West Coast of Tropical America. Part 1: Brachygnatha Oxyrhyncha[1]

JOHN S. GARTH
Allan Hancock Foundation,
University of Southern California

(Plate I; Text-figures 1 & 2)

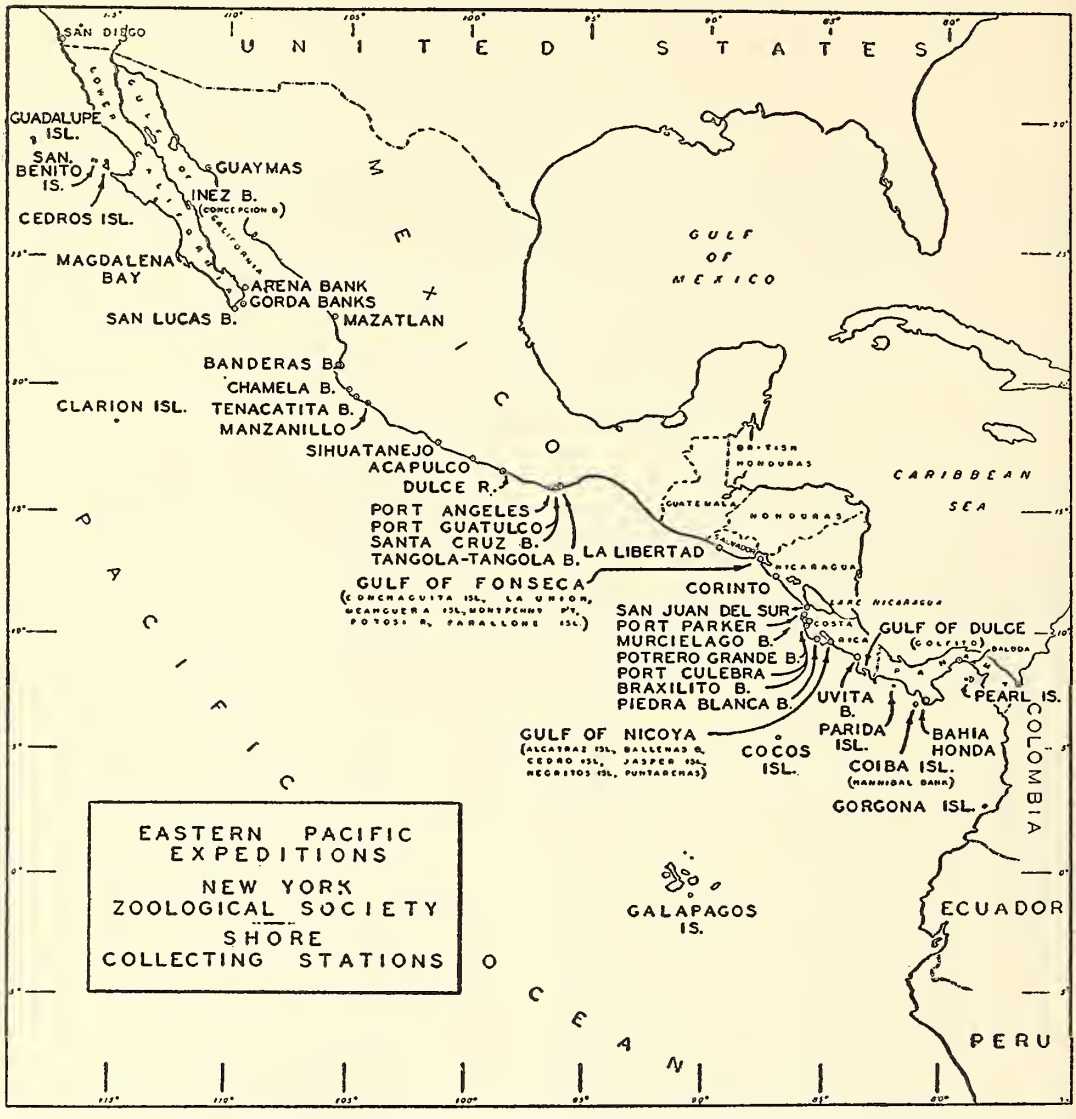
[This is the forty-fourth of a series of papers dealing with the collections of the Eastern Pacific Expeditions of the New York Zoological Society made under the direction of William Beebe. The present paper is concerned with specimens taken on the Templeton Crocker Expedition (1936) and the Eastern Pacific "Zaca" Expedition (1937-1938). For data on localities, dates, dredges, etc., refer to *Zoologica,* Vol. XXII, No. 2, pp. 33-46, and Vol. XXIII, No. 14, pp. 287-298.]

CONTENTS

INTRODUCTION

THE present study was undertaken as one of a series of reports, each devoted to the efforts of a single expedition covering an extensive but exclusive sector of the Pacific American coastline. The earliest was that of the

[1]Contribution No. 993, Department of Tropical Research, New York Zoological Society.



TEXT-FIG. 1. Shore collecting stations of the Eastern Pacific Expeditions of the New York Zoological Society. For exact locations of associated dredge stations, refer to Zoologica, vol. XXII, no 2, and vol. XXIII, no. 14.

"Zaca," under the direction of Dr. William Beebe, which in 1937 and 1938 covered the southwestern portion of North America from San Diego, California, to Balboa, Canal Zone. (See Text-fig. 1). The intertidal brachygnathous crabs of this expedition were reported on by Crane (1947). The second was that of the "Askoy" under the direction of Dr. Robert Cushman Murphy, which in 1941 covered the northwest corner of South America from Panama to Cape Santa Elena, Ecuador (Garth, 1948). The third was that of the Lund University Chile Expedition, under the direction of Professor Dr. Hans Brattström and Dr. Erik Dahl, which in 1947 and 1948 covered the southwestern portion of South America from Paita, Peru, to the Strait of Magellan (Garth, 1957). Each of the above reports, the present included, is regarded as contributing toward a series of monographs based principally but not exclusively upon the collections of the "Velero III," which from 1931 to 1941, under the direction of Capt. Allan Hancock, made annual cruises extending as far southward as San Juan Bay, Peru.

At the time of reporting on the intertidal brachygnathous crabs from the west coast of tropical America, Crane (*op. cit.*, p. 70) speci-

fically excluded "all the Portunidae, Goneplacidae, and Pinnotheridae, in spite of the fact that species of these families were occasionally taken in tidepools, coral, or in high-tide seines; the Gecarcinidae, although they occur on the fringes of both beach and mangrove areas; several *Sesarma* which proved as typically fresh-water as the Potamonidae, although they also occurred in the upper reaches of tidal streams; and all *Plagusia,* which, although rarely found in tidepools, are characteristically oceanic." The excluded families and genera, plus the dredged Majidae, Parthenopidae, Xanthidae and Grapsidae, the shore-collected members of which were included in the above-mentioned report, therefore become the subject matter of the report that follows. Since the total number of species of Brachygnatha collected other than intertidally is quite large, it has been decided to treat the Oxyrhyncha and the Brachyrhyncha in separate installments. The non-brachygnathous Dromiacea and Oxystomata will form the basis for a subsequent paper.

Ecological Considerations

Since the report on the intertidal brachygnaths emphasized the ecological and behavioristic aspects, in the observation and interpretation of which Miss Crane is without a peer among present-day carcinologists, it is only fitting that the present report on non-intertidal forms deal with these aspects also. Fortunately, the writer has been provided by Miss Crane with an extensive set of notes on color, food habits and behavior, on which he has drawn freely. It is regrettable that dredged forms are not as accessible to direct observation (or were not, before the advent of the aqua-lung) as are the intertidal forms, so that much of their ecology must be arrived at by indirect methods.

From the fact that single collections were made by shallow dredging of species normally collected in intertidal zones 3, 4 and 5 (See Crane, 1947, p. 86), namely, stones near low-tide level, tidepools and *Pocillopora* coral, it may be inferred that these biotopes extend into the subtidal or adtidal zone, lacking only the factor of exposure to air, which in the case of the lowest low-tide level may be for but a few hours each year. And from the record of the bottom types sampled it is apparent that all the basic substrata, sand, mud, gravel and rock present in the intertidal are present in the subtidal also. To cite but one example of an intertidal biotope having a subtidal extension: the mud banks of the mangrove flat not only dip below the low-tide line, but the muddy bottoms of the mangrove-fringed lagoons and esteros are covered with plant detritus that in some cases represents an almost pure culture of decaying mangrove leaves. Since multiple bottom types are often included in the same dredge haul, often with organic additions, such as shell, coral, coralline or weed, it is possible only through comparing many dredge hauls and by a process of elimination to determine the particular bottom type or types on which a given species of crab normally occurs.

A special word about the *Pocillopora* colony is needed, since Crane (*op. cit.*, p. 88), although admitting that its usual depth was from one to three or more fathoms, chose to treat it as an intertidal habitat. Exposure of beds of living coral to air, according to the writer's experience, occurs along the coastline under consideration infrequently and for periods of brief duration only, as at spring tides. At other times coral heads are obtained by grappling or diving in water of moderate depth. While the nine species mentioned by Crane (*op. cit.*, p. 89) as apparent obligate symbiotes would presumably occur in living *Pocillopora* at whatever depth it might be found, it is reasonable to suppose that the non-obligates, *i. e.*, the twelve species found by Crane in other strictly intertidal habitats, might show the vertical zonation of these other habitats, and that a new group of non-obligates might invade the *Pocillopora* below tidal levels. That this is in fact the case is suggested in the accounts of the individual species to follow. (See especially *Herbstia camptacantha* and *H. pubescens*).

Geographical Considerations

The territory explored by the "Zaca" on its 1937-1938 cruise represents but the northern and better-known half of the Panamic faunal province, which extends from Pta. Eugenia (or Pta. Entrada, *cf.* Garth, 1955), Lower California, to Pta. Santa Elena, Ecuador. It is therefore not to be expected that the number of range extensions, and particularly those southward, would approach those of the "Askoy" (Garth, 1948), which explored the lesser-known southern half in 1941. However, of those species of spider crabs heretofore believed limited to the Gulf of California, a southward range extension can be reported for *Collodes tumidus* to Manzanillo, Mexico, while of those species not earlier found north of Panama a northward extension may be noted for *Pitho quinquedentata* to northern Costa Rica and for *Collodes robsonae* to El Salvador. *Collodes tenuirostris* was collected at or near the northern limit of its range east of Cedros Island, Lower California, the northernmost locality from which specimens in this collection were obtained.

In addition to these range extensions, many intermediate localities are noted that bridge large gaps in formerly discontinuous ranges. Among

these may be mentioned a Manzanillo record for *Macrocoeloma maccullochae* that links the previously reported localities of Isabel Island, Gulf of California, and Playa Blanca, Costa Rica. The record of *Maiopsis panamensis* from Hannibal Bank is the first from the Bay of Panama since the type, although the species has been taken subsequently at Manta, Ecuador, and Clarion Island, Mexico (Garth, 1958). The inclusion of dredged material from Clarion Island collected by the "Zaca" on the 1936 Templeton Crocker Expedition does not alter the faunal list for that outpost of the Revilla Gigedo group.

None of the species of Oxyrhyncha here recorded from the west coast of Central America occurs on the east coast as well; however, numerous species of both Majidae and Parthenopidae occur on both sides of the continent as species pairs (Rathbun, 1925; Garth, 1958).

Attention has been focused on the decapod crustacean fauna of El Salvador by recent papers by Holthuis (1954) and by Bott (1955). The following species collected by the "Zaca" may be listed as new to that country: *Collodes granosus, C. robsonae, Notolopas lamellatus* and *Heterocrypta craneae.* Similarly, *Parthenope (Parthenope) hyponca* may be reported as new to Nicaragua, although the decapod crustacean fauna of that country has been comparatively better known because of the early activities of J. A. McNeil (Smith, 1871).

Systematic Considerations

The 44 species of Oxyrhyncha treated in Part 1 equals the 44 species of Brachyrhyncha to be treated in Part 2 of this paper and represents over 32 per cent. of the 135 species of Oxyrhyncha occurring on the Pacific American coast from Bering Strait to the Strait of Magellan, including the Galapagos Islands (Garth, 1958). The 35 species of Majidae represent 30 per cent. of the 118 species, the 9 species of Parthenopidae 56 per cent. of the 16 species, known to comprise the Pacific American fauna in these families. The larger percentage of parthenopids represented in the present collection reflects the more exclusively tropical distribution of that family, plus the fact that more species of Majidae than of Parthenopidae are intertidal, and so were earlier reported by Crane (1947).

Because the new species obtained by the "Velero III" from this same coastline were earlier described, in most cases as rapidly as studied (Garth, 1940, 1958), there are few novelties remaining in the present collection. However, the first male and second known specimen of *Collodes robsonae* Garth (1958) is reported and the male first pleopod figured in these pages (Text-fig. 2), while a species of *Heterocrypta* of large size, but unfortunately represented by the female sex only, is described as new to science. An earlier record of Port Parker, Costa Rica, for *Macrocoeloma maccullochae* Garth (1940) is corrected to Playa Blanca, Costa Rica.

Although Boone (1938), on the basis of a single male specimen from Hannibal Bank, Panama, anticipated the writer in synonymizing *Stenocionops triangulata* (Rathbun) with *S. macdonaldi* (Rathbun), the former the young, the latter the adult of a species currently known as *S. ovata* (Bell), the 16 adolescent specimens obtained in a single dredge haul by the "Zaca" at Manzanillo, Mexico, including both sexes in a size range from 26 to 51 mm., provide convincing evidence in support of such action. Pertinent data are presented as Table I.

In order that systematists in other groups need not scan the entire paper for the limited information germane to their studies, it should be mentioned that rhizocephalan parasites are referred to in the account of *Notolopas lamellatus,* barnacles in the account of *Herbstia pubescens* and bryozoans in the description of *Heterocrypta craneae.*

Restriction of Synonymies

In keeping with the format established in the "Askoy" Expedition report (Garth, 1948), synonymies are restricted to the original description, the first use of the name in its current combination, and the citation placing the species in the territory covered, if not included in the above two. For the preferred synonymy the reader is referred to Garth: "Brachyura of the Pacific Coast of America. Oxyrhyncha" (1958), to which the dredged Majidae and Parthenopidae reported herein must be considered as a supplement. For the convenience of those not possessing this work, reference is also made to the spider crab monograph of Rathbun (1925), and to all reported occurrences of species subsequent to this date in the eastern tropical Pacific.

Measurements

The system of measurement used is that of Garth (1958, p. 27), which differs from that of Rathbun (1925, p. 1) in but one significant aspect: the length of the doubly rostrate species of spider crab is measured along the midline from the posterior border of the carapace to an imaginary perpendicular joining the rostral spines, thereby including the rostral length in the total length of both doubly rostrate and singly rostrate species.

Acknowledgments

Gratitude is hereby expressed to Dr. William Beebe, Director Emeritus, and to Miss Jocelyn

Crane, Assistant Director, Department of Tropical Research, New York Zoological Society, for making the present collection available for study, and to Miss Crane in particular for supplying the notes on color, habit and habitat that raise the paper above the level of systematic routine and for answering promptly and cheerfully the many questions that arose as the work progressed. Appreciation also goes to the writer's associates at the Allan Hancock Foundation: to the late Dr. James W. Buchanan, who as Director of Research from 1950 to 1952 continued the earlier policy of Captain Allan Hancock of permitting staff members to devote time to collections complementing those of the "Velero III" to the enrichment of general knowledge; to Victoria Louise Smith and Janet Haig, who as Research Assistants helped with the cataloguing of the specimens; and to Dr. John D. Soule, who identified the bryozoan found on the new *Heterocrypta* species. Lastly, thanks are given to Dr. F. A. Chace, Jr., Curator of Marine Invertebrates, U. S. National Museum, for the loan of specimens of *Heterocrypta granulata* (Gibbes) for comparison.

Systematic Discussion

Tribe Brachyura
Subtribe Brachygnatha
Superfamily Oxyrhyncha
Family Majidae

Euprognatha bifida Rathbun

Euprognatha bifida Rathbun, 1893, p. 231; 1925, p. 103, pl. 34, figs. 5, 6. Crane, 1937, p. 55. Garth, 1948, p. 22; 1958, p. 61, pl. B, fig. 8; pl. 3, fig. 3.
Batrachonotus nicholsi Rathbun, 1894, p. 55; 1925, p. 127, pl. 39, figs. 5-8.

Range.—From San Benito Islands, Lower California, and Tepoca Bay, Gulf of California, Mexico, to Cape San Francisco, Ecuador. Socorro, Clarion and Cocos Islands. 0.5-90 fathoms. (Garth, 1958).

Material Examined.—Fourteen specimens from three stations:

Mexico

Clarion Island, 3 miles off Pyramid Rock, May 12, 1936, Station 163, D-2, 55 fathoms, 1 male; D-3, D-4, 50 fathoms, 1 male.

Costa Rica

Port Parker, January 20, 1938, Station 203, D-2, D-3, 10 fathoms, 7 males, 4 ovigerous females.

Panama

Bahia Honda, March 18, 1938, Station 222, D-4, 11 fathoms, 1 female.

Measurements.—Males, 5.2 to 8.0 mm., females, 4.5 to 5.8 mm. (ovigerous females, 5.4 to 5.8 mm.) in length.

Habitat.—Shelly sand, algae; dead coral, shells, gravelly mud.

Breeding.—Costa Rica in January.

Remarks.—In view of the reduction to synonymy of *Batrachonotus nicholsi* Rathbun (Garth, 1958, above), it is well to remark that the female from Bahia Honda and one of the females from Port Parker were of the type formerly referred to that genus and species.

Collodes granosus Stimpson

Collodes granosus Stimpson, 1860, p. 194, pl. 2, fig. 4. Rathbun, 1925, p. 106, pl. 36, figs. 1, 2; pl. 217, fig. 1. Garth, 1948, p. 23; 1958, p. 72, pl. E, fig. 2; pl. 3, fig. 5. Not Boone, 1930, p. 76, pl. 21, figs. A, B.

Range.—From near Punta Piaxtla, Sinaloa, Mexico, to Santa Elena Bay, Ecuador. 2-30 fathoms. (Garth, 1958)

Material Examined.—Ten specimens from five stations:

El Salvador

La Libertad, December 16, 1937, Station 198, D-1, 13 fathoms, 2 males; D-2, 14 fathoms, 1 male, 2 females.

Meanguera Island, Gulf of Fonseca, December 23, 1937, Station 199, D-1, 16 fathoms, 1 female, ovigerous.

Costa Rica

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 8 fathoms, 1 mature female, 2 young females.

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-5, D-6, 6 fathoms, 1 young female.

Measurements.—Males, 8.3 to 9.8 mm., females, 5.3 to 8.9 mm. (ovigerous female 8.5 mm.), young from 3.8 mm. in length.

Habitat.—Mud; sand, mud and crushed shell; mangrove leaves, mud, shells.

Breeding.—Gulf of Fonseca in late December.

Remarks.—The 3.8 mm. young are almost bare, while the 5.3 and 5.4 mm. females show the incipient granulation characteristic of the species.

Collodes tenuirostris Rathbun

Collodes tenuirostris Rathbun, 1893, p. 230; 1925, p. 113, pl. 37, text-fig. 35. Crane, 1937, p. 55. Garth, 1958, p. 74, pl. E, fig. 3; pl. 6, fig. 5.

Range.—From Cedros Island, west coast of Lower California, and Tepoca Bay, Gulf of California, Mexico, to Sechura Bay, Peru. 3-90 fathoms. (Garth, 1958). To 145 fathoms. (Rathbun, 1925)

Material Examined.—Eleven specimens from three stations:

Mexico

East of Cedros Island, November 10, 1937, Station 126, D-14, 45 fathoms, 1 ovigerous female.

Tangola-Tangola, December 9, 1937, Station 196, D-1, D-2, D-5, 5 fathoms, 1 young female.

Costa Rica

Off Ballenas Bay, Gulf of Nicoya, February 25, 1938, Station 213, D-15, 40 fathoms, 2 males; D-16, 45 fathoms, 4 males, 3 females (2 ovigerous).

Measurements.—Males, 14.1 to 27.5 mm., females, 13.3 to 27.4 mm. (ovigerous females, 18.0 to 27.4 mm.) in length. Since the larger of these specimens exceed the 26.2 mm. male and 21.5 mm. ovigerous female reported by Garth (1958), their measurements in mm. are given in tabular form for ease in comparison:

	Male	Female
Length of carapace	27.5	27.4
Width of carapace	22.4	22.3
Length of rostrum	2.2	2.1
Width of rostrum	2.0	1.9
Length of cheliped	31.2	26.6
Length of chela	13.8	11.2
Length of dactyl	7.5	6.2
Height of palm	4.3	2.5
Length of ambulatory legs		
First pair	53	50
Second pair	—	49
Third pair	46	45.5
Fourth pair	44	44

Color in Life.—Apparently coral pink. Completely covered, except mouth-parts, ventral side of rostrum, eye and chelae, with long, mucous-like wool of a greenish-gray mud color. Uncovered parts white spotted with chestnut; chelae entirely chestnut. Eggs bright coral red.

Behavior.—In four-by-four dish began picking off "fur," especially from frontal region, and began eating it. (Under microscope, covering looks exactly like dirty wool; when dry it is mud and glistening fibers. I am certain the whole is mucous and mud held on by hairs, which on ambulatories are very long). In aquarium, buried self in mud; just as active next morning when removed and put back in aquarium without mud. Movements, except those of chelipeds in picking off wool to eat, sluggish. (Crane, field notes).

Habitat.—Mud, algae; gravelly sand; mud.

Breeding.—West coast of Lower California in early November; Costa Rica in late February.

Remarks.—The Cedros Island record above at least duplicates, and possibly extends slightly, the northernmost record for the species.

Collodes tumidus Rathbun

Collodes tumidus Rathbun, 1898, p. 569, pl. 41, fig. 1; 1925, p. 121, pl. 40, figs. 1, 2; pl. 218, fig. 5, text-fig. 47. Crane, 1937, p. 56. Garth, 1958, p. 77, pl. E, fig. 4; pl. 3, fig. 6.

Range.—West coast of Lower California from east side of Cedros Island to Magdalena Bay; Gulf of California from Puerto Refugio, Angel de la Guarda Island, to 1¼ miles SW of Cabeza Ballena. 11-70 fathoms.

Material Examined.—Manzanillo, Mexico, November 22, 1937, Station 184, D-2, 30 fathoms, 1 male, 4 ovigerous females.

Measurements.—Male, 11.5 mm., ovigerous females, 10.5 to 11.6 mm. × 9.6 mm.

Habitat.—Gravelly sand.

Breeding.—West coast of Mexico in late November.

Remarks.—As compared to *Collodes robsonae*, *C. tumidus* is a hairy species, the ovigerous females of small size. The 11.6 mm. ovigerous female is larger than the 10.8 mm. female noted by Garth (1958).

With all previous records from either the west coast of Lower California or the Gulf of California, the record above for Manzanillo is the first from Mexico south of Cape Corrientes.

Collodes robsonae Garth

(Text-fig. 2)

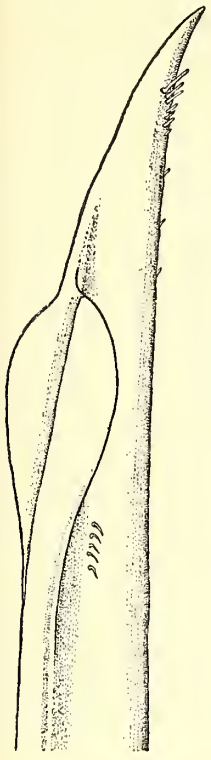
Collodes robsonae Garth, 1958, p. 78, pl. D, figs. 1-6; pl. 4, fig. 1.

Range.—Known only from the type locality, Venado, Canal Zone.

Material Examined.—Meanguera Island, Gulf of Fonseca, El Salvador, December 23, 1937, Station 199, D-1, 16 fathoms, 1 male.

Measurements.—Male specimen, length 26.0 mm., width 19.8 mm., rostrum 1.5 mm., width 2.0 mm., cheliped 26.8 mm., chela 11.0 mm., dactyl 6.8 mm., height of palm 3.1 mm., walking legs 46, 48.5, 44, and 38 mm., respectively.

Description of Male.—Differing from female as follows: Carapace lacking punctate linear striations but with corresponding inner branchial and outer intestinal regions blistered. Pits in grooves separating branchial from hepatic, gastric and cardiac regions deeper and more linear. Rostrum a little longer, extending well beyond outer antennal spine, sinus between horns V-shaped rather than U-shaped, interantennular spine visible in dorsal view. Cardiac and gastric spines longer but retaining their proportionate



TEXT-FIG. 2. *Collodes robsonae,* male, terminal portion of right first pleopod, ×90. Jens W. Knudsen, del.

lengths of 2 to 3. Horizontal row of hairs replaced by two tufts on gastric region. Postlateral marginal ridge thickened but not raised.

Basal antennal article more granulate on margins, especially the external margin opposite base of eyestalk. Rostral, internal and external basal antennal spines or teeth enclosing antennules dorsally and laterally.

Male chelipeds feeble like those of female; fingers gaping slightly for basal two-fifths, denticulate and in contact for distal three-fifths.

Male abdomen lacking a spine or tubercle on first segment, segments 4, 5 and 6 each with a low, transverse, rectangular, hairy tubercle, greatest width opposite segment 3, succeeding segments laterally concave, segments 6-7 fused, segment 7 narrowly triangular. Two diagonal, rectangular, granulate and hairy tubercles on sternum at base of chelipeds.

Tip of male pleopod slender, acuminate, lateral lobe scarcely extending beyond margins of lip enclosing aperture.

Color.—Carapace and chelipeds pale buff; ambulatories and underparts pure white; hairs all buff.

Habitat.—Sand, mud, crushed shell.

Behavior.—Used mud and mucous exactly like *Collodes tenuirostris* (which see), except that it did not attempt to pick them off. Does not bury self in mud at all. Movements sluggish. (Crane, field notes).

Remarks.—The Meanguera Island specimen is the only specimen of this species other than the type and is the first male to be recorded. Although it differs from the female in no essential other than the characters associated with its sex, these serve at once to affirm its affinities with other members of the genus *Collodes,* and to define its differences from them.

Paradasygyius depressus (Bell)

Microrhynchus depressus Bell, 1835a, p. 88; 1836, p. 42, pl. 8, figs. 2, 2d-f.

Dasygyius depressus, Rathbun, 1898, p. 570; 1925, p. 138, pl. 1; pl. 274, figs. 5-8. Boone, 1930, p. 78, pl. 22. Crane, 1937, p. 56. Garth, 1948, p. 24.

Paradasygyius depressus, Garth, 1958, p. 81, pl. E, fig. 5, pl. 4, fig. 2.

Range.—From Ensenada de San Francisco, mainland side, and outside Concepción Bay, Lower Californian side, Gulf of California, to Cape Corrientes, Colombia. 5-55 fathoms; exceptionally to 80 fathoms. (Garth, 1958).

Material Examined.—Seventeen specimens from four stations:

Mexico

Tenacatita Bay, November 21, 1937, Station 183, D-2, 30 fathoms, 1 female.

Costa Rica

Port Culebra, January 30, 1938, Station 206, D-1, 14 fathoms, 1 male.

Fourteen miles S × E of Judas Point, March 1, 1938, Station 214, D-1, D-2, 42 fathoms, 7 males, 7 females.

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-5, D-6; 6 fathoms, 1 male.

Measurements.—Males, 11.4 to 22.2 mm., females, 12.0 to 18.5 mm., and young from 4.1 mm. in length.

Color in Life.—See Crane (1937, p. 56).

Habitat.—Muddy sand; sandy mud; mud and shell; mangrove leaves.

Remarks.—The splendid series from 14 miles S × E of Judas Point lacks ovigerous females, nor does it include specimens of the size of the 27.7 mm. male and 24.8 mm. female recorded by Garth (1958).

Pyromaia tuberculata (Lockington)

Inachus tuberculatus Lockington, 1877a, p. 30.

Pyromaia tuberculata, Rathbun, 1925, p. 133, pl. 40, fig. 3; pl. 218, figs. 1-4. Crane, 1937, p. 56. Ricketts & Calvin, 1939, p. 249. Garth, 1948, p. 23; 1958, p. 85, pl. E, fig. 7; pl. 6, fig. 1.

Collodes granosus, Boone, 1930, p. 76, pl. 21, figs. A, B. Not *C. granosus* Stimpson.

Range.—From Tomales Bay, Marin County, California (extralimital), and from Monterey Bay, California, to off Cape Corrientes, Colombia, including the Gulf of California from Consag Rock south. Shore to 225 fathoms.

Material Examined.—Two specimens from two stations:

Costa Rica

Off Ballenas Bay, February 25, 1938, Station 213, D-16, 45 fathoms, 1 male.

Panama

Gulf of Chiriqui, March 13, 1938, Station 221, D-3, 35 fathoms, 1 male.

Measurements. — Larger specimen, male, length 14.9 mm., width 13.2 mm. Smaller specimen, male, length 13.8 mm., width 11.8 mm.

Habitat.—Mud; sandy mud.

Remarks.—The single male from the Bay of Panama is of the granulous variety described by Rathbun (1925, p. 136) as "Variety B." When a sufficient number of specimens from this area can be examined, it may be possible to segregate the Bay of Panama form as a subspecies, as has been done with specimens from the northern third of the Gulf of California, now known as *Pyromaia tuberculata mexicana* (Rathbun). No measurements of the Bay of Panama form are given by Garth (1958).

Inachoides laevis Stimpson

Inachoides laevis Stimpson, 1860, p. 192. Rathbun, 1925, p. 61, pl. 22, figs. 3-6 (exclusive of Atlantic material). Crane, 1937, p. 53. Garth, 1948, p. 22; 1958, p. 98, pl. E, fig. 6; pl. 6, fig. 4.

Range.—From Cedros Island and Scammon Lagoon, west coast of Lower California, and Rocky Point, Gulf of California, Mexico, to La Libertad, Ecuador. 1.5 to 56 fathoms.

Material Examined. — Nineteen specimens from five stations:

Mexico

Port Guatulco, December 6, 1937, Station 195, D-14, 4 fathoms, 1 young female.

Tangola-Tangola Bay, Station 196, December 9, 1937, D-1, D-2, D-5, 5 fathoms, 1 female; December 12, 1937, D-14, D-15, 12 fathoms, 1 ovigerous female.

Nicaragua

Corinto, Station 200, December 29, 1937, D-5, 2 fathoms, 2 ovigerous females; January 5, 1938, D-14, D-15, 1 fathom, 2 males; January 7, 1938, D-27 to D-30, 3 fathoms, 1 male.

Costa Rica

Port Parker, January 22, 1938, Station 203, D-4, 7 fathoms, 4 males, 2 females; D-6, 1 fathom, 1 ovigerous female.

Gulf of Dulce, March 9, 1938, Station 218, D-5, D-6, 6-4 fathoms, 4 males.

Measurements.—Males, 3.8 to 6.8 mm., females, 3.9 to 5.3 mm. (ovigerous females 4.0 to 5.3 mm.) in length.

Habitat.—Gravel, algae; mangrove leaves; crushed shell, rocks.

Breeding.—Mexico and Nicaragua in December, Costa Rica in late January.

Remarks.—Of small size and with a single rostral spine, this species is readily confused with both *Podochela veleronis* Garth and *Collodes tenuirostris* Rathbun. When mature males are present, as at the Costa Rican station above, the disproportionately large chelipeds with their characteristically gaping fingers make positive identification simpler.

A forthcoming report on the Caribbean collections of the "Velero III" will clarify the relationship of the Atlantic material grouped by Rathbun (1925) with Pacific under Stimpson's species.

Podochela hemphilli (Lockington)

Microrhynchus hemphillii Lockington, 1877a, p. 30.

Podochela hemphillii, Rathbun, 1898, p. 569; 1925, p. 49, pl. 18; pl. 209, fig. 2. Crane, 1937, p. 51.

Podochela hemphilli, Garth, 1958, p. 104, pl. H, fig. 6; pl. 7.

Range.—From Monterey Bay, California, and Angel de la Guarda Island, Gulf of California, Mexico, to Cabita Bay, Colombia. Clarion Island; Cocos Island. Shore to 55 fathoms (exceptionally to 90 fathoms). Rarely encountered south of the Gulf of California.

Material Examined.— Twenty-six specimens from two stations:

Mexico

SE of Cedros Island, November 10, 1937, Station 126, D-19, 25 fathoms, 1 ovigerous female.

Manzanillo, November 22, 1937, Station 184, D-2, 30 fathoms, 14 males, 11 females (10 ovigerous).

Measurements.—Males, 6.9 to 21.1 mm., females, 9.8 to 15.0 mm. (ovigerous females, 10.9 to 15.0 mm.) in length.

Habitat.—Rocks, algae; gravelly sand.

Breeding.—West coast of Lower California and west Mexico in November.

Remarks.—Although the species has been re-

ported (Garth, 1958) from Panama and even from Colombia, these records are based on admittedly inadequate material, occurrences outside of the Gulf of California to the southward being rare. It is therefore gratifying to find an established, breeding colony in Manzanillo harbor.

Podochela angulata Finnegan

Podochela angulata Finnegan, 1931, p. 617, text-fig. 3. Garth, 1948, p. 21; 1958, p. 108, pl. F, figs. 1-6; pl. H, fig. 3; pl. 8, fig. 6.

Range.—From Puerto Culebra, Costa Rica, to La Libertad, Ecuador. (Garth, 1958). 5-35 fathoms.

Material Examined.—Eight specimens from two stations:

Costa Rica

Port Parker, January 22, 1938, Station 203, D-2 or D-3, 20 fathoms, 4 males, 2 females; D-7, 9-5 fathoms, 1 male.

Fourteen miles S × E of Judas Point, March 1, 1938, Station 214, D-1, 42 fathoms, 1 male.

Measurements.—Males, 6.3 to 10.7 mm., females, 8.2 and 10.9 mm. in length.

Color in Life.—Brown or reddish.

Habitat.—Shelly sand, algae; or shelly mud.

Remarks.—Specimens from among "Velero III" collections from Port Parker were determined by M. J. Rathbun prior to 1937 as *Podochela hemphillii* (Lockington). However, subsequent studies by the writer (Garth, 1948, 1958) have shown *P. angulata* to be the common *Podochela* of the Central American coastline. The length of the dactyl of the fourth walking leg, which almost equals the length of the propodus, is diagnostic.

Podochela veleronis Garth

Podochela veleronis (MS name), Garth, 1948, p. 22.

Podochela veleronis Garth, 1958, p. 111, pl. G, figs. 1-7; pl. H, fig. 5; pl. 8, fig. 2.

Range.—From Los Frailes, Gulf of California, Mexico, to La Plata Island, Ecuador. 1-15 fathoms. (Garth, 1958).

Material Examined.—Three specimens from two stations:

Mexico

Tangola-Tangola Bay, December 12, 1937, Station 196, D-14, 5 fathoms, 1 ovigerous female.

Costa Rica

Piedra Blanca Bay, February 5, 1938, Station 208, D-?, 2-6 fathoms, 2 ovigerous females.

Measurements.—Ovigerous females, length 6.1 to 6.4 mm.

Habitat.—Crushed shell; rocks, sand, algae.

Breeding.—West Mexico in mid-December; Costa Rica in early February.

Remarks.—*Podochela veleronis* is a small species more likely to be confused with *Inachoides laevis* Stimpson than with any other *Podochela* species. Although no measurements were given by Garth (1958) to support the statement that specimens of 6 to 7 mm. were adult, the 5.7 mm. female allotype being non-ovigerous, the 6.1 to 6.4 mm. ovigerous females from Mexico and Costa Rica, above, support this contention, as does a 6.0 mm. ovigerous female from among "Velero III" collections.

Podochela vestita (Stimpson)

Podonema vestita Stimpson, 1871, p. 97.

Podochela vestita, A. Milne Edwards, 1879, p. 195. Rathbun, 1925, p. 42, pl. 14. Crane, 1937, p. 52, pl. 1. Garth, 1948, p. 21; 1958, p. 121, pl. H, fig. 7; pl. 8, fig. 3.

Range.—From Hughes Point, Lower California, and Rocky Point, Sonora, Mexico, to Gorgona Island, Colombia. Socorro Island. 2-30 fathoms. (Garth, 1958)

Material Examined. — Corinto, Nicaragua, January 7, 1938, Station 200, D-27 to D-30, 3 fathoms, 1 female (soft body).

Measurements. — Female specimen, length 12.8 mm.

Habitat.—Mangrove leaves.

Remarks. — Taken with *Inachoides laevis* Stimpson.

Podochela ziesenhennei Garth

Podochela ziesenhennei Garth, 1940, p. 58, pl. 13, figs. 1-6; 1958, p. 127, pl. H, fig. 9; pl. 8, fig. 5.

Range.—From Tenacatita Bay, Mexico, to Salango Island, Ecuador. Shore to 10 fathoms. (Garth, 1958).

Material Examined.—Port Guatulco, Mexico, December 6, 1937, Station 195, D-14, 4 fathoms, 1 female.

Measurements. — Female specimen, length 10.6 mm.

Habitat.—Coral bottom.

Remarks.—The female from Port Guatulco is post ovigerous and is nearly as large as an 11.0 mm. female from Acapulco recorded by Garth (1958).

Stenorynchus debilis (Smith)

Leptopodia debilis Smith, 1871, p. 87.

Stenorynchus debilis, Rathbun, 1898, p. 568; 1925, p. 18, pls. 4, 5, text-fig. 4. Finnegan, 1931, p. 617. Crane, 1937, p. 50. Steinbeck & Ricketts, 1941,

p. 465. Garth, 1948, p. 20; 1958, p. 130, pl. B, fig. 7; pl. 9. Buitendijk, 1950, p. 271.

Range.—From Magdalena Bay, west coast of Lower California, and Patos Anchorage, Gulf of California, Mexico, to Valparaiso, Chile. Revilla Gigedo, Galapagos and Cocos Islands. Shore to 78 fathoms.

Material Examined. — Thirty-six specimens from eleven stations:

Mexico

Sulphur Bay, Clarion Island, May 11, 1936, Station 163, D-1, 20 fathoms, 1 young.

Port Guatulco, December 4, 1937, Station 195, D-1, 2.5 fathoms, 2 males, 3 females; D-16, 10 fathoms, 2 males, 2 females.

Tangola-Tangola Bay, December 12, 1937, Station 196, D-15, 5 fathoms, 1 male, 3 females.

Nicaragua

Corinto, January 7, 1938, Station 200, D-27 to D-30, 3 fathoms, 1 male.

Costa Rica

Port Parker, harbor, January 16, 1938, 6 males, 3 females (1 ovigerous), 2 young.

Port Parker, January 22, 1938, Station 203, D-4, 7 fathoms, 1 male; D-7, 9-5 fathoms, 1 young; D-12, 2 fathoms, 1 male, 1 ovigerous female; D-13, 7-9 fathoms, 1 male.

Port Culebra, January 30, 1938, Station 206, D-1, 14 fathoms, 1 female.

Off Ballenas Bay, Gulf of Nicoya, Station 213, D-17, 35 fathoms, 1 male.

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-4, 6 fathoms, 1 male.

Panama

Off Panama, March 13, 1938, Station 221, D-3, 35 fathoms, 1 female, post-ovigerous.

Bahia Honda, March 18, 1938, Station 222, D-4, 11 fathoms, 1 male.

Measurements.—Males, 10.3 to 33.1 mm., females 10.1 to 32.8 mm. (ovigerous females 8.5 [without rostrum] and 21.1 mm.), young from 3.9 mm. in length.

Color in Life.—Typical coloring, [*cf.* Crane, 1937] but pale. (Of Port Guatulco specimen).

Habitat.—Sand, shell and gravel with algae often present; mud with mangrove leaves frequently present.

Remarks.—In measuring the extensive series, specimens under 10.0 mm. were arbitrarily considered as young, although sex could be determined in 9 mm. males. The fact that the rostrum is frequently broken in this species makes it impossible to give the total length of the smaller of two ovigerous females, or the absolute length of a large male from the Gulf of Nicoya that measured 30 mm. without the terminal portion. Complete measurements of a 38 mm. male and 34 mm. female are given in tabular form by Garth (1958).

Pitho picteti (Saussure)

Othonia picteti Saussure, 1853, p. 357, pl. 13, fig. 2.

Pitho picteti, Rathbun, 1923, p. 635; 1925, p. 359, pl. 130, figs. 2, 3; pl. 252, fig. 1. Finnegan, 1931, p. 624. Glassell, 1934, p. 454. Crane, 1937, p. 59. Steinbeck & Ricketts, 1941, p. 466. Garth, 1958, p. 166, pl. J, fig. 1; pl. 17, fig. 1.

Pitho quinquedentata, Boone, 1929, p. 563, figs. 1a-b. (Not *P. quinquedentata* Bell.)

Range.—From Scammon Lagoon, west coast of Lower California, and off Cape Tepoca, Gulf of California, Mexico, to Saboga Island, Perlas Islands, Panama. Shore to 45 fathoms.

Material Examined. — Eighteen specimens from four stations:

Mexico

Port Guatulco, December 4, 1937, Station 195, D-1, 2.5 fathoms, 1 ovigerous female; D-3, 3.5 fathoms, 1 young; D-4, 4.5 fathoms, 1 ovigerous female; D-10, 4 fathoms, 1 male, 3 young.

Nicaragua

Corinto, December 29, 1937, Station 200, D-5, 2 fathoms, 1 ovigerous female.

Costa Rica

Port Parker, January 22, 1938, Station 203, D-6, 1 fathom, 1 ovigerous female; D-11, 2-4 fathoms, 1 ovigerous female, 4 young.

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 4-10 fathoms, 2 males, 1 female, 1 young.

Measurements.—Males, 8.6 to 10.8 mm., females, 8.3 to 12.1 mm. (ovigerous females 10.0 to 12.1 mm.), young from 4.7 mm. in length.

Breeding.—Mexico and Nicaragua in December, Costa Rica in January.

Color in Life.—Gray with pink longitudinal median stripe, dark gray below. Eggs brick red. (Port Guatulco specimen.)

Habitat.—Sand, algae, crushed shell, dead coral; mangrove leaves; rock, gravel, dead coral; mud, sand, crushed shell.

Pitho quinquedentata Bell

Pitho quinquedentata Bell, 1835b, p. 172. Rathbun, 1925, p. 361, pl. 250, figs. 1-4, text-fig. 117a. Garth, 1958, p. 170, pl. J, fig. 2; pl. 18, fig. 1.

Range.—Bay of Panama; Galapagos Islands; ?Peru. (Rathbun, 1925). Shore to 20 fathoms. (Garth, 1958).

Material Examined.—Three specimens from three stations:

Costa Rica

Port Parker, January 22, 1938, Station 203, D-10, 6-2.5 fathoms, 1 young female.

Piedra Blanca, February 5, 1938, Station 208, D-?, depth?, 1 female.

Panama

Bahia Honda, March 18, 1938, Station 222, D-1, 3 fathoms, 1 female.

Measurements.—Female specimens, length 7.3 to 8.2 mm.

Habitat.—Rocks; rocks, sand and algae; rock, dead coral.

Remarks.—With specimens from Bahia Honda collected by the "Velero III" constituting the only record for the species north of Cape Mala, it is gratifying to have this record confirmed and the range of the species extended to northern Costa Rica by the "Zaca."

Sphenocarcinus agassizi Rathbun

Sphenocarcinus agassizi Rathbun, 1893, p. 231; 1925, p. 188, pl. 63; pl. 223, figs. 1, 2. Faxon, 1895, p. 7, pl. 1, figs. 3, 3a. Crane, 1937, p. 58. Garth, 1946, p. 379, pl. 63, fig. 2; 1958, p. 217, pl. O, fig. 1; pl. 25, fig. 1.

Range.—From off Cape Tepoca, Gulf of California, Mexico, to Bahia Honda, Panama. Galapagos Islands. 14-90 fathoms.

Material Examined.—Two specimens from two stations:

Mexico

Gorda Banks, November 13, 1937, Station 150, D-27, 60 fathoms, 1 female.

Panama

Hannibal Bank, March 20, 1938, Station 224, D-2, D-3, 35 fathoms, 2 males, 3 females.

Measurements.—Males, 14.3 to 29.6 mm., females, 11.8 to 21.2 mm. in length.

Color in Life.—Pale tan with a dark brown stripe on each side of median line of carapace. (Of Gorda Banks specimen.)

Habitat.—Sand at Gorda Banks; rocks, mud and dead coral at Hannibal Bank.

Supplementary Descriptive Note.—A feature not mentioned by Rathbun is the dorsal flattening of the lateral teeth. This is most apparent on the first, or hepatic tooth, each succeeding lateral tooth showing it to a lesser degree.

Remarks.—It is of interest to note the close correspondence in size of the largest male and female collected by the "Zaca" with the 30.0 mm. male and 23.1 mm. female collected by the "Velero III." Neither male is as large as the 39 mm. male collected by the "Albatross" (Faxon, 1895).

Although the species was early reported from "Bay of Panama" by Faxon (1895, p. 7), the actual locality was Cocos Island, Costa Rica. It remained for the "Velero III" to record the species from the Panamanian mainland at Bahia Honda, a record confirmed by the "Zaca" collection at Hannibal Bank.

Pelia tumida (Lockington)

Pisoides? tumidus Lockington, 1877a, p. 30.

Pelia tumida, Holmes, 1900, p. 35. Rathbun, 1925, p. 281, pl. 99, figs. 2, 3. Garth, 1958, p. 271, pl. Q, fig. 1; pl. 31, fig. 2, text-fig. 6a.

Range.—From Santa Cruz Island, California, and Rocky Point, Gulf of California, to Petatlan Bay, Guerrero, Mexico. Shore to 55 (possibly 70) fathoms.

Material Examined.—SE of Cedros Island, Lower California, Mexico, November 10, 1937, Station 126, D-19, 25 fathoms, 1 female.

Measurements.—Female specimen, length 7.3 mm.

Habitat.—Rocks and algae.

Remarks.—In this species, which has for its provenance southern California, Lower California, the Gulf of California and the northwest Mexican coast, the rostral horns have their outer margins parallel or divergent, while of the basal antennal article only the anteroexternal angle bearing the spine is visible in dorsal view.

Pelia pacifica A. Milne Edwards

Pelia pacifica A. Milne Edwards, 1875, p. 73, pl. 16, figs. 3-3c. Rathbun, 1925, p. 283, pl. 98, fig. 1; pl. 99, fig. 1. Crane, 1947, p. 71. Garth, 1958, p. 274, pl. Q, figs. 2-4; pl. 31, fig. 3, text-figs. 6b, 6c.

Range.—From Manzanillo, Mexico, to Zorritos Light, Peru. Intertidal, occasionally to 2 fathoms.

Material Examined.—Three specimens from two stations:

Nicaragua

Corinto, January 5, 1938, Station 200, D-14, D-15, 1-3 fathoms, 2 females (one ovigerous).

Costa Rica

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 8 fathoms, 1 male.

Measurements.—Male specimen 5.25 mm.,

females 4.9 and 6.5 mm. (ovigerous female 4.9 mm.) in length.

Habitat.—Mangrove leaves and mud.

Breeding.—Nicaragua in early January.

Remarks.—Since *Pelia pacifica* was reported by Crane (1947) from the intertidal zone of Corinto, Nicaragua, and at Jasper Island and Uvita, Costa Rica, it was thought that dredged specimens from these two countries might prove to be the foregoing *P. tumida,* which occurs in more northerly waters in depths to 55 fathoms. Comparison with the Cedros Island, Mexico, specimen, and with typical *P. tumida* from southern California, however, revealed these constant differences: the rostral horns of the Central American specimens invariably have their outer margins converging, while of the basal antennal article fully half, rather than just the anteroexternal angle, is visible in dorsal view.

Notolopas lamellatus Stimpson

Notolopas lamellatus Stimpson, 1871, p. 97. Rathbun, 1925, p. 287, pl. 81; pl. 238, fig. 1, text-fig. 95. Garth, 1948, p. 26; 1958, p. 295, pl. Q, fig. 8; pl. 33, fig. 1.

Pelia orbiculata Finnegan, 1931, p. 621, text-fig. 4.

Range.—From Rocky Point, Sonora, Mexico, to La Libertad, Ecuador. Shore to 20 (exceptionally 55) fathoms. (Garth, 1958).

Material Examined.—Twenty-five specimens from four stations:

El Salvador

La Libertad, December 16, 1937, Station 198, D-1, 13 fathoms, 1 male, 1 ovigerous female; D-2, 14 fathoms, 1 ovigerous female.

La Union, Gulf of Fonseca, December 27, 1937, Station 199, D-8, 6 fathoms, 1 ovigerous female; D-21, 3 fathoms, 1 ovigerous female.

Nicaragua

Corinto, December 29, 1937, Station 200, D-5, 2 fathoms, 2 males, 2 females (1 ovigerous), 1 young; D-5, 2 fathoms, 1 young; D-27, D-30, 3 fathoms, 6 males, 5 females (1 ovigerous).

Costa Rica

Piedra Blanca Bay, February 5, 1938, Station 208, D-?, depth?, 2 males, 1 young.

Measurements.—Males 5.6 to 13.6 mm., females 6.0 to 19.3 mm. (ovigerous females 8.3 to 19.3 mm.), young from 4.2 mm. in length.

Habitat.—Mud; mangrove leaves; rocks, algae.

Breeding.—El Salvador in mid- to late December; Nicaragua in late December and early January.

Remarks.—Specimens from El Salvador lack the interorbital spine and have a broad exorbital lobe. Two ovigerous females are exceptionally large and show divergence of rostral horns and a cardiac tubercle. Specimens from Corinto, Nicaragua, have the interorbital spine, and one female carries a rhizocephalan parasite. Specimens are variously decorated with algae, hydroids and bryozoans.

The 19.3 mm. ovigerous female above is larger than the 15.5 mm. ovigerous female reported by Garth (1958).

Herbstia camptacantha (Stimpson)

Herbstiella camptacantha Stimpson, 1871, p. 94.

Herbstia camptacantha, A. Milne Edwards, 1875, p. 78, pl. 18, figs. 3-3e. Rathbun, 1925, p. 294, pl. 105, figs. 1, 2; pl. 240, figs. 9-13. Garth, 1958, p. 301, pl. S, fig. 1; pl. 34, fig. 1.

Range.—From San Pedro Bay, Sonora, to Tangola-Tangola Bay, Guerrero, Mexico. Shore to 4.5 fathoms.

Material Examined.—Sixteen specimens from three stations:

Mexico

Sihuatanejo, November 24, 1937, 3 males, 5 ovigerous females.

Acapulco Beach, November 26-28, 1937, 1 ovigerous female.

Port Guatulco, December 6, 1937, Station 195, D-14, 4 fathoms, 1 male; D-15, 1.5 fathoms, 4 males, 2 ovigerous females.

Measurements.—Males 7.0 to 14.0 mm., ovigerous females 11.0 to 18.2 mm. in length.

Breeding.—Mexico in late November and early December.

Habitat.—Coral.

Remarks.—Although *Pocillopora* coral was included by Crane (1937, p. 88) as an intertidal habitat because of its partial exposure during spring tides, its normal depth range is adtidal, or from one to four fathoms. Included among the dredgings from Port Guatulco were coral heads that yielded a species of *Herbstia, H. camptacantha,* not encountered in coral obtained by other methods and from shallower depths, where the common *Herbstia* was *H. tumida* (Stimpson).

The 18.2 mm. ovigerous female above is larger than the 14.3 mm. ovigerous female reported by Garth (1958), and would be even longer were it not for the fact that the rostrum is broken off near the base. For this reason complete measurements are not given.

Herbstia pubescens Stimpson

Herbstia pubescens Stimpson, 1871, p. 92. Rathbun, 1925, p. 302. Garth, 1948, p. 27; 1958, p. 308, pl. S, fig. 7; pl. 34, fig. 3.

Range.—From Manzanillo, Mexico, to La Plata Island, Ecuador. (Garth, 1948). Shallow water to 3.5 fathoms. (Garth, 1958).

Material Examined.—Four specimens from two stations:

Costa Rica

Port Parker, January 19, 1938, abajo coral, 1 male, 1 ovigerous female.

Port Culebra, January 31, 1938, *Pocillopora,* 1 male, 1 female.

Measurements.—Males 9.1 and 15.5 mm., females 8.0 and 16.8 mm., the latter ovigerous. The larger male exceeds the 14.0 mm. male reported by Garth (1958), and measures as follows: length 15.5 mm., width 11.8 mm., rostrum 1.7 mm., width 2.3 mm., cheliped 18.5 mm., chela 11.9 mm., dactyl 5.3 mm., height of palm 4.4 mm. The 16.8 mm. ovigerous female also exceeds the 14.6 mm. ovigerous female reported by Garth (1948) but is so overgrown with barnacles that complete measurements cannot be given.

Habitat.—*Pocillopora* coral.

Breeding.—Costa Rica in January.

Remarks.—That *Herbstia pubescens* is a constant concommitant of the coral colony throughout at least the southern portion of its range, from Puerto Culebra, Costa Rica, southward, is apparent from the collections of the "Askoy," the "Zaca" and the "Velero III." Previous to their advent, it had been unknown in these waters, Stimpson's type locality having been Manzanillo, to the north. The female specimen from Port Parker carries a tremendous weight of *Balanus* for its size.

Herbstia tumida (Stimpson)

Herbstiella tumida Stimpson, 1871, p. 95.

Herbstia tumida, A. Milne Edwards, 1875, p. 79. Rathbun, 1925, p. 229, pl. 105, figs. 5, 6. Finnegan, 1931, p. 623. Crane, 1937, p. 59; 1947, p. 72. Garth, 1948, p. 27; 1958, p. 313, pl. R, figs. 1-5; pl. S, figs. 3, 4, 6; pl. 34, fig. 4.

Range.—From Arena Bank, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. Clarion and Cocos Islands. Shore to 15 (exceptionally to 50) fathoms. (Garth, 1958).

Material Examined.—From Jasper Island, Gulf of Nicoya, Costa Rica, February 23, 1938, 2 males, 1 ovigerous female.

Measurements.—Males 7.1 and 9.0 mm., female 9.8 mm. in length.

Breeding.—Costa Rica in late February.

Habitat.—[*Pocillopora*] coral.

Remarks.—Since no depth is given with the specimens above, their separation for purposes of report from the immature female from Bahia Honda, Panama (Crane, 1947, p. 72) would appear to serve no useful purpose, particularly in view of the fact that the species has been reported by Crane (1937, p. 59) from *Pocillopora ligulata* in 2.5 fathoms in the Gulf of California, albeit with a question mark.

The 9.8 mm. ovigerous female from the Gulf of Nicoya is larger than either the 8.6 mm. non-ovigerous or 8.3 mm. ovigerous females reported from the mainland, but not as large as the 10.8 mm. ovigerous female reported from Clarion Island by Garth (1958).

Lissa aurivilliusi Rathbun

Lissa aurivilliusi Rathbun, 1898, p. 575, pl. 41, fig. 4; 1925, p. 333, pl. 246, fig. 2. Crane, 1937, p. 59. Garth, 1946, p. 384, pl. 65, figs. 3, 4; 1958, p. 335, pl. T, fig. 8; pl. 33, fig. 4.

Range.—From Santa Maria Bay, Lower California, and Puerto Refugio, Angel de la Guarda Island, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. Galapagos Islands. 2 to 35 (exceptionally 70) fathoms. (Garth, 1958).

Material Examined.—Port Parker, Costa Rica, January 20, 1938, Station 203, D-2, 10 fathoms, 1 male.

Measurements.—Male specimen, length 9.0 mm.

Habitat.—Shelly sand, algae.

Remarks.—The species varies greatly in the degree of development of the rostral horns and the lateral extent of the branchial regions.

Maiopsis panamensis Faxon

Maiopsis panamensis Faxon, 1893, p. 151; 1895, p. 13, pl. 2. Rathbun, 1925, p. 338, pl. 247. Garth, 1958, p. 342, pl. U, figs. 3, 3a; pl. 38, figs. 1, 2; pl. 39, fig. 1.

Range.—Clarion Island; Bay of Panama to Ecuador. 48-182 fathoms.

Material Examined.—Hannibal Bank, Panama, March 20, 1938, Station 224, D-1, 40 fathoms, 1 female.

Measurements. — Female specimen, length 62.2 mm., width including lateral spines 62.7 mm., without spines 52.2 mm. Complete measurements of a larger female from Clarion Island and of a large male from Ecuador are given by Garth (1958).

Habitat.—Rocks, dead coral.

Remarks.—The "Zaca" record above is the

first from Panama since the type specimen, a male, was collected by the "Albatross" in 1891. In the interim, a second male was collected at Manta, Ecuador, by Capt. Paessler and a female at Clarion Island, Mexico, by the "Velero III." (Garth, 1958). The Hannibal Bank female is 10 mm. shorter than the Clarion Island specimen.

Ala cornuta (Stimpson)

Anaptychus cornutus Stimpson, 1860, p. 184, pl. 2, figs. 1, 1a, 1b. Rathbun, 1925, p. 378, pl. 134, figs. 4, 5; pl. 254, fig. 1; text-fig. 122. Steinbeck & Ricketts, 1941, p. 467. Crane, 1947, p. 72. Garth, 1948, p. 28.

Ala cornuta, Garth, 1958, p. 349, pl. V, figs. 1, 2; pl. 39, fig. 2.

Range.—From Cholla Bay, Gulf of California, Mexico, to Port Utria, Colombia. Shore to 12 fathoms.

Material Examined.—Port Parker, Costa Rica, January 22, 1938, Station 203, D-10, 6-2.5 fathoms, 1 young; D-11, 2-4 fathoms, 1 male.

Measurements.—Male specimen, length 12.0 mm., young specimen, length 5.0 mm.

Habitat.—Rocks.

Remarks.—The species is reported from the intertidal zone by Crane (1947, p. 72) at two Mexican, five Costa Rican and one Panamanian localities, at extreme low-tide level and in *Pocillopora* coral. It is therefore not surprising that it should be taken also in shallow dredging. The 5 mm. young has long rostral spines and might easily be mistaken for another species.

Mithrax (Mithrax) sinensis clarionensis Garth

Mithrax (Mithrax) clarionensis Garth, 1940, p. 63, pl. 15, figs. 1-3.

Mithrax (Mithrax) sinensis clarionensis, Garth, 1958, p. 363, pl. V, fig. 6; pl. 41, fig. 3.

Range.—Restricted to Clarion Island, Mexico. 15-57 fathoms.

Material Examined.—Sulphur Bay, Clarion Island, Mexico, May 11, 1936, Station 163, D-1, 20 fathoms, 1 male.

Measurements.—Male specimen, length 9.4 mm., width 8.8 mm.

Habitat.—Not given.

Remarks.—While the isolation of Clarion Island from Gulf of Californian *Mithrax* (*Mithrax*) *sinensis* Rathbun is amply evident in the finer tuberculation of the former, their male first pleopods are identical.

Mithrax (Mithrax) pygmaeus Bell

Mithrax pygmaeus Bell, 1835b, p. 172; 1836, p. 55, pl. 11, figs. 3, 3f-h. Finnegan, 1931, p. 624. Crane, 1947, p. 73.

Mithrax (Mithrax) pygmaeus, Rathbun, 1925, p. 406, pl. 262, figs. 1-4. Garth, 1948, p. 29; 1958, p. 364, pl. V, fig. 7; pl. 41, fig. 4.

Range.—From Isabel Island, Mexico, to La Plata Island, Ecuador. Socorro Island; Galapagos Islands. Low-tide level to 25 fathoms.

Material Examined.—Five specimens from three stations:

Mexico

Port Guatulco, December 5, 1937, Station 195, D-8, 6 fathoms, 1 ovigerous female; D-9, 7 fathoms, 1 male.

Tangola-Tangola Bay, December 12, 1937, Station 196, D-14, D-15, 5 fathoms, 1 female.

Costa Rica

Port Parker, January 22, 1938, Station 203, D-5, 3 fathoms, 1 ovigerous female; D-10, 6-2.5 fathoms, 1 female.

Measurements.—Male, 7.8 mm., females, 5.2 to 6.3 mm. in length; ovigerous females same. Largest specimen, male, length 7.8 mm., width including spines 8.4 mm., female, length 6.3, width 6.8 mm.

Habitat.—Sand, algae, crushed shell; dead coral; rocks.

Breeding.—Mexico in early December; Costa Rica in late January.

Remarks.—The species was recorded also by Crane (1947) from the intertidal, where it occurs in the *Pocillopora* colony. All females were either with ova or had borne ova previously. The long bare chelipeds of the male are characteristic of the species.

Mithrax (Mithraculus) denticulatus Bell

Mithrax denticulatus Bell, 1835b, p. 172; 1836, p. 54, pl. 11, figs. 2, 2c-e. Crane, 1947, p. 73.

Mithrax areolatus Lockington, 1877b, p. 71.

Mithrax (Mithraculus) denticulatus, Rathbun, 1925, p. 428; pl. 154, figs. 2, 3. Garth, 1958, p. 372, pl. V, fig. 9; pl. 42, fig. 2. Buitendijk, 1950, p. 274.

Range.—From San Diego, California (extralimital), and Agua Verde Bay, Gulf of California, Mexico, to Manta Bay, Ecuador. Intertidal, occasionally dredged to 13 fathoms. Absent from the Revilla Gigedo Islands, and from Galapagos Islands, where it is replaced by *Mithrax* (*Mithraculus*) *nodosus* Bell.

Material Examined.—Port Guatulco, Mexico, December 4, 1937, Station 195, D-4, 4.5 fathoms, 1 young male.

Measurements.—Young male, length 5.9 mm.

Habitat.—Sand, crushed shell, algae.

Remarks.—Eighty-six specimens were recorded by Crane (1947, p. 73) from four Mexican, four Costa Rican and one Panamanian

localities, where they occurred under stones at extreme low tide (zone 3), in tidepools among weed (zone 4), and in *Pocillopora* coral (zone 5). The above specimen, dredged in 4.5 fathoms, merely adds another zone, the subtidal or adtidal, which, except for exposure, cannot differ greatly from zones 3 and 4.

For a discussion of color variability, and for reasons for including *Mithrax areolatus* Lockington (1877) in the synonymy, see Crane (*op. cit.*).

Microphrys platysoma (Stimpson)

Milnia platysoma Stimpson, 1860, p. 180.

Microphrys platysoma, A. Milne Edwards, 1875, p. 62. Rathbun, 1925, p. 479, pl. 176, figs. 1, 2; text-fig. 140. Crane, 1937, p. 63; 1947, p. 74. Steinbeck & Ricketts, 1941, p. 467, pl. 32, fig. 6. Garth, 1946, p. 405, pl. 68, figs. 3, 4; 1948, p. 30; 1958, p. 392, pl. W, fig. 5; pl. 43, fig. 3.

Range.—From 10 miles west of Punta Malarrimo, Lower California, and Puerto Refugio, Angel de la Guarda Island, Gulf of California, Mexico, to Punta Santa Elena, Ecuador. Clarion and Socorro Islands; Galapagos Islands. Intertidal to 40 (not 70) fathoms. (Garth, 1958).

Material Examined.—Jasper Island, Gulf of Nicoya, Costa Rica, February 23, 1938, 1 male.

Measurements.—Male specimen, length 13.1 mm., width 10.6 mm.

Habitat.—Not given.

Remarks.—Previously reported from the intertidal of Clarion Island, Costa Rica and Panama, where it occurs under stones at low-tide level, in tidepools and in *Pocillopora* coral, zones 3, 4, and 5. (Crane, 1947).

Microphrys triangulatus (Lockington)

Mithraculus triangulatus Lockington, 1877b, p. 73.

Microphrys triangulatus, Rathbun, 1898, p. 578; 1925, p. 505, pl. 177, text-fig. 147. Garth, 1946, p. 403, pl. 63, fig. 6; 1958, p. 395, pl. W, figs. 6, 9; pl. 43, fig. 4.

Range.—From off Concepción Bay, Gulf of California, Mexico, to Bahia Honda, Panama. Galapagos Islands. Intertidal to 44 fathoms. (Garth, 1958).

Material Examined.—Fifty-three specimens from a single station:

Mexico

Port Guatulco, Station 195, December 4, 1937, D-3, 3.5 fathoms, 1 male, 1 female; D-4, 4.5 fathoms, 14 males, 7 females (4 ovigerous); D-5 to D-8, 5 fathoms, 14 males, 12 females (8 ovigerous); December 6, 1937, D-10, 4 fathoms, 2 females; D-14, 4 fathoms, 2 males.

Measurements.—Males, 5.3 to 12.1 mm., females, 6.2 to 10.0 mm. (ovigerous females 7.1 to 10.0 mm.) in length.

Color in Life.—Purple with olive buff pile. Eggs raspberry.

Habitat.—Sand, crushed shell, algae, dead coral.

Breeding.—Mexico in early December.

Remarks.—The Port Guatulco specimens are important from the standpoint of distribution, the species having been recorded outside the Gulf of California and on the mainland only at Acapulco, Mexico, and Bahia Honda, Panama.

Microphrys branchialis Rathbun

Microphrys branchialis Rathbun, 1898, p. 577, pl. 41, fig. 5; 1925, p. 502, pl. 176, figs. 5, 6; pl. 270, fig. 1; text-fig. 143. Crane, 1937, p. 63. Schmitt, 1939, p. 9. Garth, 1958, p. 398, pl. W, figs. 7, 8; pl. 44, fig. 1.

Range.—From Dewey Channel, west coast of Lower California, and from Angeles Bay, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. 5-50 fathoms.

Material Examined.—Six specimens from two stations:

Mexico

SE of Cedros Island, November 10, 1937, Station 126, D-19, 25 fathoms, 1 ovigerous female.

Costa Rica

Port Parker, January 20, 1938, Station 203, D-2, D-3, 10-12 fathoms, 2 males; D-7, 9-5 fathoms, 2 males, 1 ovigerous female.

Measurements.—Males, 5.6 to 10.5 mm., females, 8.6 and 9.1 mm. (ovigerous females same) in length.

Color in Life.—Carapace deep purple; legs and under parts brown; eggs dark purple. (Of Cedros Island specimen.)

Habitat.—Rocks, algae; shelly sand, algae, shelly mud.

Breeding.—Lower California in early November; Costa Rica in late January.

Remarks.—The intermittent and sometimes overlapping ranges of this species and the related *Microphrys triangulatus* are discussed by Garth (1958, p. 400). Unlike the companion species, *M. branchialis* is never collected intertidally.

Stenocionops ovata (Bell)

Pericera ovata Bell, 1835b, p. 173; 1836, p. 60, pl. 12, figs. 5, 5o-q.

Stenocionops triangulata, Rathbun, 1925, p. 461, pl. 165, fig. 1; pl. 266, fig. 1. Garth, 1946, p. 401, pl. 67, figs. 1, 2; pl. 68, fig. 2.

TABLE I. *Stenocionops ovata* (BELL)

Sex	Length	Number of median carapace spines*				
		gastric	genital	cardiac	intestinal	total
♂	51.0 mm	4	1	3	1	9
♂	42.9 mm	2(1)2	1	3	1	9(1)
♀	42.6 mm	4	0	3	1	8
♀	41.4 mm	4	1	3	1	9
♂	41.2 mm	4	1	3	1	9
♀	39.0 mm	4	1	3	1	9
♂	37.2 mm	4	1	1(2)	1	7(2)
♀	36.1 mm	4	0	3	1	8
♀	34.4 mm	4	(1)	1(2)	1	6(3)
♂	34.0 mm	4	0	1	1	6
♀	33.1 mm	4	0	3	1	8
♂	32.0 mm†	4	0	2	1	7
♀	31.8 mm	4	1	3	1	9
♂	29.8 mm	4	0	1(2)	1	6(2)
♂	28.5 mm	4	1	2	1	8
♂	26.2 mm	4	0	1(1)	1	6(1)

* Incipient or reduced spines in parentheses † Broken rostrum

Stenocionops ovata, Rathbun, 1910, p. 574. Boone, 1938, pp. 201, 220, pls. 79, 80. Garth, 1958, p. 405, pl. Y, fig. 2; pl. 44, fig. 2.

Stenocionops macdonaldi, Rathbun, 1925, p. 460, pl. 268. Crane, 1937, p. 62.

Range.—From off Abreojos Point, west coast of Lower California, and Tiburon Island, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. Galapagos Islands. 8-150 fathoms. (Garth, 1958).

Material Examined. — Manzanillo, Mexico, November 22, 1937, Station 184, D-2, 30 fathoms, 9 males, 7 females, all adolescent.

Measurements.—See Table I.

Color in Life.—Dusty pink.

Habitat.—Gravelly sand.

Remarks.—The above series of 16 specimens of 26 to 51 mm. length is the one that should have been used in synonymizing *Stenocionops triangulata* (Rathbun, 1892) with *S. macdonaldi* (Rathbun, 1892), as suggested by Crane (1937). Fortunately, the action taken by Boone (1938, p. 220) on the strength of one large male dredged at Hannibal Bank, Panama, by the "Alva" is supported by this supplementary material. Needed now is a similar series from the Galapagos Islands to demonstrate conclusively Boone's correctness in synonymizing both to *Stenocionops ovata* (Bell), which has a Galapagan type locality.

From the above table it will be seen that, with the exception of the 42.9 mm. male with an incipient fifth spine, the number of gastric spines remains constant at four, as does the intestinal spine at one. The genital spine is present with but one exception in specimens over 37 mm. The greatest irregularity occurs with the cardiac spines, the posterior two of which are often poorly developed or even wanting in specimens under 39 mm.

Macrocoeloma maccullochae Garth

Macrocoeloma maccullochae Garth, 1940, p. 65, pl. 16, figs. 1-4; 1958, p. 413, pl. Y, fig. 4; pl. 46, fig. 1.

Range.—From Isabel Island, Mexico, to Santa Elena Bay, Ecuador. 10 to 30 fathoms. (Garth, 1958).

Material Examined.—Four specimens from two stations:

Mexico

Manzanillo, November 22, 1937, Station 184, D-2, 30 fathoms, 1 male.

Costa Rica

Port Parker, Station 203, January 20, 1938, D-2, 10 fathoms, 1 male; January 22, 1938, D-4, 7 fathoms, 1 young; D-5, 3 fathoms, 1 young male.

Measurements. — Largest specimen, male: length 41.3 mm., width including lateral spines 28.1 mm., without spines 24.8 mm., rostrum 10.2 mm., width 3.7 mm., cheliped 48.6 mm., chela 22.0 mm., dactyl 7.3 mm., height of palm 3.3 mm., walking legs 42.0, 33.5, 28.5, and 25.5 mm., respectively.

Habitat.—Gravelly sand; shelly sand, with algae; shells, dead coral.

Remarks.—The Manzanillo male is the largest specimen on record, measuring a full 10 mm.

longer than the male holotype. Its rostrum is perfect, whereas the tip of the rostrum of the holotype is broken. The narrowness of the young, mentioned by Garth (1958) as characteristic of the species, is noticeable in males of 7.6 and 10.5 mm. in the present series. While the species was earlier reported from Port Parker (Garth, 1940), this proved to be an error for Playa Blanca, Costa Rica (Garth, 1958). The Manzanillo record is new and bridges the gap between Costa Rica and Isabel Island, Mexico.

Hemus finneganae Garth

Hemus finneganae Garth, 1958, p. 422, pl. X, figs. 1-6; pl. Y, fig. 7; pl. 47, fig. 2.

Range.—From Angel de la Guarda Island, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. Revilla Gigedo Islands. Shore to 32 fathoms. (Garth, 1958).

Material Examined. — Eighteen specimens from three stations:

Mexico

Port Guatulco, December 4, 1937, Station 195, D-4, 4.5 fathoms, 1 female.

Costa Rica

Port Parker, January 22, 1938, Station 203, D-5, 3 fathoms, 2 males, 3 ovigerous females; D-6, 1 fathom, 3 specimens; D-10, 6-2.5 fathoms, 1 male, 1 ovigerous female; D-11, 2-4 fathoms, 2 ovigerous females.

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 4-10 fathoms, 1 male, 4 females (2 ovigerous).

Measurements.—Males, 6.6 to 8.0 mm., females, 5.5 to 7.8 mm. (ovigerous females, 5.5 to 7.8 mm.) in length. Complete measurements of the largest male, which exceeds by 1.3 mm. the 6.7 mm. holotype (Garth, 1958), are as follows: length 8.0 mm., width 6.9 mm., rostrum 0.9 mm., width 1.35 mm., cheliped 5.3 mm., chela 2.0 mm., dactyl 0.9 mm., ambulatory legs 6.9, 6.8, 6.2, and 4.2 mm., respectively. The feeble cheliped should be noted.

Habitat.—Sand, algae and crushed shell; rocks and gravel; mud; dead coral.

Breeding.—Costa Rica in late January and early February.

Remarks.—It is unusual to find a new species the exclusive occupant of so large a territory as is *Hemus finneganae*. The reason is that the companion species, *H. analogus* Rathbun, is not the Pacific analogue of the Atlantic *H. cristulipes*, as Rathbun supposed, but a Gulf of California endemic species that apparently comes no further south than Tenacatita Bay, Mexico. *H. finneganae* ranges both north and south of the more strictly limited *H. analogus*, and represents *H. cristulipes* in the Bay of Panama.

Family PARTHENOPIDAE

Parthenope (Parthenope) hyponca (Stimpson)

Lambrus hyponcus Stimpson, 1871, p. 100.

Parthenope (Parthenope) hyponca, Rathbun, 1925, p. 514, pl. 275, figs. 4-6. Garth, 1948, p. 30; 1958, p. 436, pl. Z_1, figs. 1, 1a; pl. 48, fig. 1.

Range.—From Mazatlan, Mexico, to Punta Santa Elena, Ecuador. 3-25 fathoms. (Garth, 1958).

Material Examined.—Corinto, Nicaragua, Station 200, December 29, 1937, D-7, 2 fathoms, 1 young male; January 5, 1938, D-14, D-15, 1-3 fathoms, 1 young male.

Measurements.—Young males, 4.7 × 4.8 mm. and 5.0 × 4.9 mm., respectively.

Habitat.—Mangrove leaves. One specimen had coarse, dark sand grains adhering.

Remarks.—The above record is the first from Nicaragua.

Parthenope (Platylambrus) exilipes (Rathbun)

Lambrus (Parthenolambrus) exilipes Rathbun, 1893, p. 234.

Parthenope (Platylambrus) exilipes, Rathbun, 1925, p. 523, pls. 184, 185; pl. 277, figs. 1, 2. Crane, 1937, p. 64. Garth, 1946, p. 409, pl. 69, fig. 2; 1958, p. 439, pl. Z_1, figs. 3, 3a; pl. 48, fig. 2.

Range.—From San Domingo Point, Lower California, and Boca de la Trinidad, Gulf of California, Mexico, to Lobos de Afuera Island, Peru. Socorro Island; Galapagos Islands. 12-95 fathoms; occasionally taken intertidally. (Garth, 1958).

Material Examined. — Twenty-six specimens from four stations:

Mexico

Gorda Banks, November 13, 1937, Station 150, D-27, 60 fathoms, 1 female.

Manzanillo, November 22, 1937, Station 184, D-2, 30 fathoms, 2 males, 6 females (3 ovigerous).

Costa Rica

Fourteen miles S × E of Judas Point, March 1, 1938, Station 214, D-1, 42 fathoms, 1 male; D-3, D-4, 50-61 fathoms, 3 males, 10 females (1 ovigerous).

Panama

Hannibal Bank, March 20, 1938, Station 224, D-1, D-2, 35-40 fathoms, 1 male, 1 female, 1 young.

Measurements.—Males, 11.1 mm. to 33.1 mm., females, 13.8 to 24.5 mm., ovigerous females, 18.8 to 23.5 mm., young from 8.3 mm. in length. The largest male exceeds the 15.6 mm. male reported by Garth (1958) and measures as follows: length 33.1 mm., width including spines 49.5 mm., rostrum 2.5 mm., width 1.7 mm., cheliped 112 mm., chela 55.5 mm., dactyl 18.5 mm., height of palm 8.3 mm.

Color in Life.—Pale tan, except inside of chelipeds, which are salmon pink. A band of deep purple across inside of base of chelae. Posterior anterolateral line pale pink. (Of Gorda Banks female.) Carapace dark brown except white cardiac and intestinal regions. Inner margins of chelipeds salmon orange. (Of Manzanillo specimen.)

Habitat.—Sand, gravelly sand; mud, with shell or rocks; rocks, mud and dead coral.

Breeding.—Mexico in late November; Costa Rica in early March.

Remarks.—The size of the specimens is remarkable. The measured male above had a span of 230 mm., or 9 inches, with chelipeds fully extended.

Daldorfia garthi Glassell

Daldorfia garthi Glassell, 1940, p. 67, pl. 17, figs. 1-11. Garth, 1946, p. 412, pl. 55, figs. 1-11; 1958, p. 455, pl. Z_2, figs. 7, 7a; pl. 51, fig. 2. Crane, 1947, p. 74.

Range.—From Cape San Lucas, Lower California, Mexico, to Octavia Bay, Colombia. Galapagos Islands. Shore to 12 fathoms. (Garth, 1958).

Material Examined.—Two specimens from as many stations:

Mexico

Port Guatulco, December 4, 1937, Station 195, D-4, 4.5 fathoms, 1 male.

Costa Rica

Port Culebra, January 29, 1938, coral, 1 female.

Measurements. — Male, length 10.7 mm., width 15.5 mm. Female, length 13.1 mm., width 20.0 mm.

Color in Life.—Apparently purple underneath growth of white bryozoans. (Of Port Guatulco male).

Habitat.—Sand, algae, crushed shell; coral.

Remarks.—This species was the only parthenopid taken intertidally by the "Zaca," a large, worn male having been reported by Crane (1947, p. 74) from under a rock at extreme low-tide level (zone 3) at Port Parker, Costa Rica.

Solenolambrus arcuatus Stimpson

Solenolambrus arcuatus Stimpson, 1871, p. 101. Rathbun, 1925, p. 538. Finnegan, 1931, p. 625. Garth, 1946, p. 413, pl. 69, figs. 3, 4; 1948, p. 31; 1958, p. 459, pl. Z_3, figs. 9, 9a; pl. 52, fig. 1.

Range.—From Tepoca Bay, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. Socorro Island; Galapagos Islands. 1.5 to 60 fathoms. (Garth, 1958).

Material Examined.—Twenty-one specimens from two stations:

Costa Rica

Port Parker, January 22, 1938, Station 203, D-4, 7 fathoms, 1 male.

Colombia

Gorgona Island, March 31, 1938, Station 232, D-1, 2-8 fathoms, 10 males, 4 females, 6 young.

Measurements. — Largest specimen, male, length 10.8 mm., width 13.9 mm. Largest female, length 8.5 mm., width 10.7 mm. Males, 6.8 to 10.8 mm., females, 7.3 to 8.5 mm. Young from 5.0 mm.

Habitat.—Gravel, algae; sand.

Remarks.—The 10.8 mm. male above is larger than the 10.7 mm. male reported by Garth (1958), although not so large as an 11.2 mm. female.

Leiolambrus punctatissimus (Owen)

Parthenope punctatissima Owen, 1839, p. 81, pl. 24, fig. 4.

Leiolambrus punctatissimus, Holmes, 1900, p. 46. Rathbun, 1925, p. 543, pl. 198, text-fig. 149. Finnegan, 1931, p. 626. Garth, 1948, p. 32; 1958, p. 462, pl. Z_3, figs. 10, 10a; pl. 52, fig. 2.

Range.—From off Point Tosco, Lower California, and off Guaymas, Gulf of California, Mexico, to Salango Island, Ecuador. (Garth, 1958). Not California (Owen). 12-45 fathoms.

Material Examined.—Four specimens from two stations:

Costa Rica

Off Ballenas Bay, Gulf of Nicoya, February 25, 1938, Station 213, D-11, D-12, D-13, 35 fathoms, 1 male, 2 females.

Panama

Gulf of Chiriqui, March 13, 1938, Station 221, D-4, 38 fathoms, 1 young female.

Measurements.—Largest specimen, female, length 18.1 mm., width 24.2 mm. Largest male, length 14.5 mm., width 20.1 mm. Females, 9.0 to 18.1 mm. in length.

Habitat.—Mud; sandy mud.

Remarks.—The 18.1 mm. female is larger than the 11.3 mm. female, but not as large as the 19.6 mm. male reported by Garth (1958).

Mesorhoea belli (A. Milne Edwards)

Solenolambrus bellii A. Milne Edwards, 1878, p. 163, pl. 29, figs. 6-6d.

Mesorhoea bellii, Rathbun, 1925, p. 548, pl. 201; pl. 280, figs. 1-4. Crane, 1937, p. 65. Garth, 1946, p. 414, pl. 69, figs. 5, 6; 1948, p. 32.

Mesorhoea belli, Garth, 1958, p. 465, pl. Z_3, figs. 11, 11a; pl. 54, fig. 1.

Range.—From San Juanico Bay, Lower California, and Georges Island, Gulf of California, Mexico, to off Esmeraldas, Ecuador. Galapagos Islands. 10-40 (exceptionally to 90) fathoms. (Garth, 1958).

Material Examined.—14 miles S × E of Judas Point, Costa Rica, March 1, 1938, Station 214, D-3, 50 fathoms, 1 female.

Measurements.—Female specimen, length 14.8 mm., width 19.5 mm.

Habitat.—Mud.

Remarks.—The 14.8 mm. female above corresponds to the largest (14.9 mm.) specimen reported by Garth (1958), also a female.

Cryptopodia hassleri Rathbun

Cryptopodia hassleri Rathbun, 1925, p. 554, pl. 202, figs. 1, 2. Garth, 1948, p. 32; 1958, p. 471, pl. Z_4, figs. 15, 15a; pl. 54, fig. 2.

Range.—From Santa Maria Bay, west coast of Lower California, and Puerto Refugio, Angel de la Guarda Island, Gulf of California, Mexico, to Santa Elena Bay, Ecuador.

Material Examined.—Three specimens from two stations:

Costa Rica

Port Parker, January 20, 1938, Station 203, D-2, D-3, 12 fathoms, 1 male, 1 female.

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 4-10 fathoms, 1 male.

Measurements.—Largest specimen, male, length 6.25 mm., width 9.75 mm. Female specimen, length 4.1 mm., width 5.7 mm. Smaller male, length 4.3 mm., width 6.4 mm.

Habitat.—Mud; shelly mud.

Remarks.—The Gulf of Nicoya male is perfect in every detail and would have provided a better photographic illustration than that in Garth (1958, pl. 54, fig. 2), which is of an imperfect specimen.

Heterocrypta macrobrachia Stimpson

Heterocrypta macrobrachia Stimpson, 1871, p. 103. Rathbun, 1925, p. 558, pl. 203, figs. 3, 4; pl. 282, figs. 4, 5. Garth, 1948, p. 33; 1958, p. 474, pl. Z_4, figs. 16, 16a; pl. 55, fig. 1.

Range.—From Santa Maria Bay, west coast of Lower California, and Rocky Point, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. 2-26 fathoms. (Garth, 1958).

Material Examined.—Twenty-five specimens from two stations:

El Salvador

Meanguera Island, Gulf of Fonseca, December 23, 1937, Station 199, D-1, 16 fathoms, 3 males, 6 females (4 ovigerous).

Costa Rica

Cedro Island, Gulf of Nicoya, Station 213, D-1 to D-10, 4-10 fathoms, 10 males, 6 females (3 ovigerous).

Measurements.—Males, 4.0 to 10.0 mm., females, 4.8 to 7.1 mm., ovigerous females, 5.1 to 7.1 mm.

Color in Life.—Very variable. Young female entirely brownish-gray, speckled on carapace and chelipeds with medium brown. Ovigerous female with orange eggs similarly marked, but with white longitudinal streaks on carapace, and posterior part of carapace cream. Chelipeds broadly banded with brown and speckled brownish-gray and pure cream. Young male almost all buffy with gastric and anterolateral regions alone speckled and dark in the usual way; chelipeds buff. (Crane, field notes).

Habitat.—Mud; shelly mud. The broken bold markings make the crabs look like a group of broken shells exactly matching the bottom.

Remarks.—Crane (in field notes) suggests that the broken markings are a holdover from a shallow-water adaptation, not now of much use to the crabs because of the dark environment in which they are found.

Heterocrypta craneae, new species

(Plate 1)

Type.—Female holotype, A.H.F. No. 376, and female paratype, N.Y.Z.S. No. 37,708, from La Unión, Gulf of Fonseca, El Salvador, December 27, 1937, "Zaca" Station 199, D-8, 6 fathoms, mud and mangrove leaves.

Measurements.—Female holotype, length 19.7 mm., width 28.4 mm., front 1.8 mm., width 2.9 mm., fronto-orbit 5.2 mm., major cheliped 35.5 mm., chela 19.6 mm., dactyl 9.5 mm., height of palm 8.0 mm. Female paratype, length 18.4 mm., width 24.5 mm.

Diagnosis.—Carapace nearly one and one-half

times as wide as long, margins crenulate. Posterolateral margin between branchial ridge and lateral angle concave. Branchial ridges uniting in paired tubercles defining gastric ridge. Cardiac region high, domed.

Description.—Carapace wide, length over two-thirds width, and high, especially gastric and cardiac regions. Branchial ridges paralleling crenulate anterolateral margins except on gastric region, there terminating in two berried tubercles, side by side. From these a longitudinal crest of flattened tubercles running forward to inner margin of orbit. A large, domed elevation surmounting cardiac region; gastric region equally elevated. Surface smooth and punctate, margins crenulate, a closed fissure one-third of the way between outer orbital and lateral angle. Posterolateral margin between lateral angle and branchial ridge deeply indented. Posterior margin concealing legs except for merus of last pair. Rostrum advanced, narrow, sides arcuate, tip triangulate.

Chelipeds unequal, short and heavy as compared to *Heterocrypta macrobrachia* Stimpson, but not as compared to *H. granulata* (Gibbes). Upper surface of manus, but not of merus, dilated at mid-point, margins of both segments and of carpus crenulate. Fingers of major chela gaping broadly at base, lower margin of pollex straight or slightly sinuous, upper margin with a basal ridge and distal tooth; dactylus down-curving, inner margin denticulate, three granulate superior crests. Fingers of minor chela slenderer, deflexed, meeting without a gape, tips crossing. Margins of walking legs minutely denticulate.

External maxillipeds with ischium grooved, merus granulate and punctate, subquadrate emargination at anterointernal angle not completely filled by palp, leaving a small opening.

Female abdomen with a transverse row of granules on segments two and three, sparsely granulate elsewhere, segment seven narrowly triangular. Male of the species unknown.

Remarks.—The discovery by the "Zaca" of a new species of *Heterocrypta* from the Gulf of Fonseca calls for a reexamination of the species relationships of this amphi-American genus. Apparently *H. craneae* is the true representative in the eastern Pacific of the short-armed *H. granulata* (Gibbes) of the western Atlantic, from which it differs most conspicuously by its larger size and indented posterolateral margin. According to this analysis, the writer's earlier assignment of *H. colombiana* Garth (1940, p. 72) to the role of Pacific analogue of *H. granulata* was both premature and in error. *H. colombiana,* which reexamination shows to lack ventrally the granular ridge connecting the anterior corner of the buccal frame with the base of the cheliped, might better have been referred to *Cryptopodia* Milne Edwards.

The two specimens from La Unión are encrusted with a bryozoan identified by Dr. John D. Soule of the Allan Hancock Foundation as *Membranipora savarti* (Audouin, 1826).

I take pleasure in naming this distinctive parthenopid species for Miss Jocelyn Crane, M. Sc., Assistant Director of the Department of Tropical Research of the New York Zoological Society, through whose diligence as a collector and skill as an observer many hitherto obscure facts concerning crabs have been made known.

Literature Cited

Bell, T.

1835a. On *Microrhynchus,* a new genus of triangular crabs. Proc. Zool. Soc. London, p. 88.

1835b. Some account of the Crustacea of the coasts of South America, with descriptions of new genera and species; founded principally on the collections obtained by Mr. Cuming and Mr. Miller. (Tribus 1, Oxyrhynchi). Proc. Zool. Soc. London, pp. 169-173.

1836. Some account of the Crustacea of the coasts of South America, with descriptions of new genera and species: founded principally on the collections obtained by Mr. Cuming and Mr. Miller. Trans. Zool. Soc. London, vol. 2, pp. 39-66, pls. 8-13.

Boone, Lee

1929. A collection of brachyuran Crustacea from the Bay of Panama and the fresh waters of the Canal Zone. Bull. Amer. Mus. Nat. Hist., vol. 58, pp. 561-583, text-figs. 1-18.

1930. Scientific results of the cruises of the yachts "Eagle" and "Ara," 1921-1928, William K. Vanderbilt, commanding. Crustacea: Stomatopoda and Brachyura. Bull. Vanderbilt Mar. Mus., vol. 2, pp. 1-228, pls. 1-74.

1938. Scientific results of the world cruises of the yachts "Ara," 1928-1929, and "Alva," 1931-1932, "Alva" Mediterranean Cruise, 1933, and "Alva" South American Cruise, 1935, William K. Vanderbilt, commanding. Part V, Crustacea. Bull. Vanderbilt Mar. Mus., vol. 7, pp. 197-281, pls. 71-109.

Bott, R.

1955. Dekapoden (Crustacea) aus El Salvador. 2. Litorale Dekapoden, ausser *Uca.* Senckenbergiana, Biologica, vol. 36, pp. 45-70, pls. 3-8, text-figs. 1-7.

Buitendijk, Alida M.

1950. Note on a collection of Decapoda Brachyura from the coasts of Mexico, including the description of a new genus and species. Zool. Meded. Rijksmus. Natuur. Hist. Leiden, vol. 30, pp. 269-282, pl. 10, text-fig. 1.

Crane, Jocelyn

1937. The Templeton Crocker Expedition. III. Brachygnathous crabs from the Gulf of California and the west coast of Lower California. Zoologica, vol. 22, pp. 47-78, pls. 1-8.

1947. Intertidal brachygnathous crabs from the west coast of tropical America with special reference to ecology. Zoologica, vol. 32, pp. 69-95, text-figs. 1-3.

Faxon, W.

1893. Reports on the dredging operations off the west coast of Central America to the Galapagos, to the west coast of Mexico, and in the Gulf of California . . . by the U. S. Fish Commission steamer "Albatross," during 1891 . . . VI. Preliminary descriptions of new species of Crustacea. Bull. Mus. Compar. Zool. Harvard, vol. 24, pp. 149-220.

1895. Reports on an exploration off the west coasts of Mexico, Central and South America, and off the Galapagos Islands . . . by the U. S. Fish Commission steamer "Albatross," during 1891 . . . XV. The stalk-eyed Crustacea. Mem. Mus. Compar. Zool. Harvard, vol. 18, pp. 1-292, pls. A-K, 1-56.

Finnegan, Susan

1931. Report on the Brachyura collected in Central America, the Gorgona and Galapagos Islands, by Dr. Crossland on the 'St. George' Expedition to the Pacific, 1924-25. Jour. Linn. Soc. London, Zool., vol. 37, pp. 607-673, text-figs. 1-6.

Garth, J. S.

1940. Some new species of brachyuran crabs from Mexico and the Central and South American mainland. Allan Hancock Pacific Exped., vol. 5, no. 3, pp. 53-127, pls. 11-26.

1946. Littoral brachyuran fauna of the Galapagos Archipelago. Allan Hancock Pacific Exped., vol. 5, no. 10, pp. (iv) 341-601, pls. 49-87, text-fig. 1.

1948. The Brachyura of the "Askoy" Expedition with remarks on carcinological collecting in the Panama Bight. Bull. Amer. Mus. Nat. Hist., vol. 92, art. 1, pp. 1-66, pls. 1-8, text-figs. 1-5.

1955. The case for a warm-temperature marine fauna on the west coast of North America. *In* Essays in the natural sciences in honor of Captain Allan Hancock, pp. 19-27. Univ. So. Calif. Press.

1957. Reports of the Lund University Chile Expedition 1948-1949. No. 29. The Crustacea Decapoda Brachyura of Chile. Lunds Univ. Årsskr., n. s., Avd. 2, vol. 53, no. 7, pp. 1-127, pls. 1-4, text-figs. 1-11.

1958. Brachyura of the Pacific coast of America. Oxyrhyncha. Allan Hancock Pacific Exped., vol. 21, pp. (vi) 1-859, pls. A-Z, Z_1-Z_4, 1-55, text-figs. 1-10. In 2 parts.

Glassell, S. A.

1934. Some corrections needed in recent carcinological literature. Trans. San Diego Soc. Nat. Hist., vol. 7, pp. 453-454.

1940. *In* Garth, J. S.: Some new species of brachyuran crabs from Mexico and the Central and South American mainland. Allan Hancock Pacific Exped., vol. 5, no. 3, pp. 67-70, pl. 17.

Holmes, S. J.

1900. Synopsis of the California stalk-eyed Crustacea. Occas. Papers Calif. Acad. Sci., vol. 7, pp. 1-262, pls. 1-4.

Holthuis, L. B.

1954. On a collection of decapod Crustacea from the republic of El Salvador (Central America). Zool. Verhandel. Rijksmus. Natuur. Hist. Leiden, no. 23, pp. 1-43, pls. 1-2, text-figs. 1-15.

Lockington, W. N.

1877a. Remarks on the Crustacea of the Pacific coast, with descriptions of some new species. Proc. Calif. Acad. Sci., vol. 7, pp. 28-36.

1877b. Remarks on the Crustacea of the Pacific coast of North America, including a catalogue of the species in the museum of the California Academy of Sciences, San Francisco. Proc. Calif. Acad. Sci., vol. 7, pp. 63-78.

Milne Edwards, A.

1873-1880. Études sur les Xiphosures et les Crustacés de la région mexicaine. Mission Scientifique au Mexique et dans l'Amérique centrale, pt. 5, pp. 368, pls. 1-61. Paris.

Owen, R.

1839. Crustacea. *In* The Zoology of Beechey's Voyage. . . . Pp. 77-92, pls. 24-28. London.

Rathbun, Mary J.

1892. Catalogue of the crabs of the family Periceridae in the U. S. National Museum. Proc. U. S. Natl. Mus., vol. 15, pp. 231-277, pls. 28-40.

1893. Scientific results of explorations by the

U. S. Fish Commission steamer Albatross. XXIV. Descriptions of new genera and species of crabs from the west coast of North America and the Sandwich Islands. Proc. U. S. Natl. Mus., vol. 16, pp. 223-260.

1894. Notes on the crabs of the family Inachidae in the United States National Museum. Proc. U. S. Natl. Mus., vol. 17, pp. 43-75, pl. 1.

1898. The Brachyura collected by the U. S. Fish Commission steamer Albatross on the voyage from Norfolk, Virginia, to San Francisco, California, 1887-1888. Proc. U. S. Natl. Mus., vol. 21, pp. 567-616, pls. 41-44.

1923. Scientific results of the expedition to the Gulf of California . . . by the U. S. Fisheries steamship "Albatross," in 1911 . . . XIII. The brachyuran crabs collected by the U. S. Fisheries steamer "Albatross" in 1911, chiefly on the west coast of Mexico. Bull. Amer. Mus. Nat. Hist., vol. 48, pp. 619-637, pls. 26-36, text-figs. 1-7.

1925. The spider crabs of America. U. S. Natl. Mus. Bull. 129, pp. 1-613, pls. 1-283, text-figs. 1-153.

Ricketts, E. F., & J. Calvin

1939. Between Pacific tides. Pp. (xxii) 320, pls. 1-46, text-figs. 1-112. Stanford.

Saussure, H. de

1853. Description de quelques Crustacés nouveaux de la côte occidentale du Mexique. Rev. Mag. Zool., ser. 2, vol. 5, pp. 354-368, pls. 12-13.

Schmitt, W. L.

1939. Decapod and other Crustacea collected on the Presidential Cruise of 1938 (with introduction and station data). Smithson. Misc. Collect., vol. 98, no. 6, pp. 1-29, pls. 1-3.

Smith, S. I.

1871. List of the Crustacea collected by J. A. McNiel in Central America. Rpt. Peabody Acad. Sci. for 1869 and 1870, pp. 87-98.

Steinbeck, J., & E. F. Ricketts

1941. Sea of Cortez. A leisurely journal of travel and research. Pp. 598, pls. 1-40, 2 charts. New York.

Stimpson, W.

1860. Notes on North American Crustacea, in the museum of the Smithsonian Institution. No. II. Ann. Lyceum Nat. Hist. New York, vol. 7, pp. 176-246, pls. 2, 5.

1871. Notes on North American Crustacea, in the museum of the Smithsonian Institution. No. III. Ann. Lyceum Nat. Hist. New York, vol. 10, pp. 92-136.

EXPLANATION OF THE PLATE

Plate I

Heterocrypta craneae, new species

Fig. 1. Female holotype, dorsal view.

Fig. 2. Female holotype, ventral view. (Carapace and chelipeds heavily encrusted with the bryozoan, *Membranipora savarti*)

FIG. 1

FIG. 2

NON-INTERTIDAL BRACHYGNATHOUS CRABS FROM THE WEST COAST OF TROPICAL AMERICA

8

Stomach Contents and Organ Weights of Some Bluefin Tuna, *Thunnus thynnus* (Linnaeus), near Bimini, Bahamas[1]

Louis A. Krumholz

Department of Biology, University of Louisville, Louisville, Kentucky

THIS paper records some observations on the kinds and amounts of foods taken from the stomachs of bluefin tuna, together with measurements of the relative weights of some of the viscera of those tuna. In addition, observations were made on the state of development of the gonads and whether or not they appeared to be spent.

Stomach Contents

Although the bluefin tuna is highly prized as a sport fish in the western North Atlantic Ocean, as attested by the many tuna tournaments held in the Bahamas and along the eastern coast of the United States, there are few published records of the different foods eaten by this species. The account in which the greatest number of stomachs was examined is that of Crane (1936) who listed eight separate organisms taken from 34 stomachs. Of those stomachs, five were empty, and of the 29 that remained, 26 contained from 1 to 38 specimens of hake (*Merluccius bilineatus*), four contained seaweed, three contained one or two squids each, two contained large numbers of adult krill (*Meganycitiphanes norvegica*), one contained a single clupeid fish, one contained three clupeid fish of the same kind but different from the one just mentioned, one contained four rosefish (*Sebastes marinus*), and one contained a single specimen of the belonid fish, *Tylosurus marinus.*

In their account of the fishes of the Gulf of Maine, Bigelow & Schroeder (1953) stated that bluefin tuna in that area prey on smaller fishes, especially those of the schooling kinds, that are most abundant locally. They also stated that in the Gulf of Maine the bluefin tuna destroys great numbers of herring and mackerel.

The only published record of foods of the bluefin tuna from the Bahamas is that of deSylva (1956), in which he stated that stomachs of that species taken near Cat Cay, about 12 miles south of Bimini, contained freshly digested squids, along with squid beaks and the radulae of bottom-dwelling snails.

The bluefin tuna referred to in this paper were made available by the Bimini Big Game Fishing Club which sponsored the Bimini Tuna Tournament, May 19-23, 1956. All seven tuna taken during the tournament were turned over to me for study purposes as soon as they were brought to the dock and officially weighed in. The weight of the first tuna brought in is not known, the fish having been mutilated by a shark.

As soon as the fish were weighed, their bellies were split open and the gonads were examined. There were two females and five males, and the condition of the ovaries indicated that both females were mature and probably had spawned within a relatively short time before being caught. The testes of all the males contained mature sperm and their condition indicated that those individuals had spawned already or were in spawning condition at the time of capture.

The stomachs were excised and the identifiable food organisms were removed and preserved in 10 percent. formalin and taken to the Lerner Marine Laboratory for identification. The use of those facilities is hereby acknowledged. Also, I am grateful to Donald deSylva and Gilbert Voss of the University of Miami Marine Laboratory for assistance in identifying the food organisms.

A total of 661 organisms, referable to seven different species, were taken from the stomachs

[1]Contribution No. 25 (New Series) from the Department of Biology, University of Louisville, Louisville 8, Ky.

of the seven tuna (Table 1). However, not more than four nor fewer than two kinds of organisms were found in any one stomach. The greatest number of organisms found in any one stomach was 275 and the smallest number was four. The most numerous organism was the porcupine fish, *Diodon hystrix*; a total of 560 individuals had been eaten by six of the seven tuna examined. Each porcupine fish was about the size of an English walnut and weighed about 5 grams. They were obviously young of the year, and at that size and age, porcupine fish are known to aggregate at or near the surface. The second most abundant food item was the salp, *Pyrosoma atlantica gigantea,* and four of the tuna had eaten 87 individuals. These salps were four to five inches long and about an inch in diameter. Entire vertebral columns, together with attached crania, of five small, eel-like fish were found in three stomachs. Although the species of fish to which the vertebral columns belonged were not identified, it is believed that they all were of the same species. The columns ranged in length from 6 to 8.5 inches. In addition to the above-mentioned salp, there were three specimens of an obviously different, but unidentified, species of salp in one stomach. Also, one stomach contained the remains of four portunid craps (*Portunus* sp.), and the beak of a small, unidentified octopus. A large plant leaf taken from one stomach was shaped somewhat like a new moon and was about five inches long.

From these data, although they admittedly are meager, several interesting inferences may be drawn. These are (1) the absence of any of the finned fishes such as the herrings, mackerels or mullets, and (2) the presence of large numbers of pelagic porcupine fish in the tuna stomachs.

In her account of the foods of the bluefin tuna near Portland, Maine, Crane (1936) noted that hake from 8 to 13 inches long constituted the principal food item. Similarly, Bigelow & Schroeder (1953) inferred that the principal food of the bluefin tuna was the herring and mackerel or similar species that were locally abundant. Although deSylva (1956) found only the remains of squids and snails in the stomachs of bluefin tuna near Cat Cay, Bahamas, he listed squids, flying fishes, sardines, herring and krill as preferred food items. In the present study, although no such fish were found in the stomachs, the tuna taken during the tournament were caught with baits of finned fishes such as mullet (*Mugil* sp.) that were 12 to 15 inches long. From this information, it is obvious that the bluefin tuna has an extremely varied diet and that the selected foods consist of animals that live at many different depths of the ocean, from the surface to the bottom. This statement is reminiscent of the remarks of Beebe (1936) about the food habits of the blackfin tuna, *Parathunnus atlanticus,* taken near Bermuda.

> "When I examined the stomach of the first of these tunas, I realized that the contents were alien to the shallow waters of Bermuda along

Table 1. Length, Weight and Sex of Seven Bluefin Tuna Caught near Bimini, Bahamas, together with a List of Organisms Taken from Each Stomach

Tuna No.	Total Length (in.)	Weight (lbs.)	Sex	Stomach Contents
1	92	ca. 470	Male	63 *Diodon hystrix* 1 eel-like vertebral column
2	93	470	Female	82 *Diodon hystrix*
3	100	565	Male	242 *Diodon hystrix* 31 *Pyrosoma atlantica gigantea* 1 eel-like vertebral column 1 unidentified plant leaf
4	92	450	Male	29 *Diodon hystrix* 13 *Pyrosoma altantica gigantea*
5	88	361	Male	63 *Diodon hystrix* 12 *Pyrosoma atlantica gigantea* 3 unidentified salps
6	95	370	Male	81 *Diodon hystrix* 31 *Pyrosoma atlantica gigantea* 3 eel-like vertebral columns
7	97	562	Female	4 crabs, *Portunus* sp. 1 beak of unidentified octopus

shore, and yet had nothing in common with the fauna of the deeper, offshore areas. And I will here anticipate another discovery which was emphasized again and again, that these great fish had almost without exception been feeding close to the bottom. Somehow, I had never visualized these swift, pelagic beings as searching over, around and perhaps in the gorges and arches of the eroded limestone. But for that matter I had never thought to find such small, spiny organisms as squilla larvae dominant in their diet."

Beebe listed a total of 1,616 organisms referable to 23 separate species in the stomachs of 18 blackfin tuna taken in September near Bermuda. In the same paper, he listed 22 different kinds of organisms, with a total of 209 individuals, in the stomachs of eight yellowfin tuna, *Neothunnus argentivittata*. However, six of the yellowfin tuna were taken near St. Lucia and one was taken near Bermuda.

In the present study, porcupine fish made up 85 percent. of the total number, and well over 90 percent. of the total weight, of the food organisms recovered from the bluefin tuna stomachs. Thus, for the moment, porcupine fish were the principal item in the diet. This observation falls in line with that of Bigelow & Schroeder (1953) in which locally abundant fishes are eaten most frequently. Although adult porcupine fish are fairly common near Bimini, the occurrence of such large numbers of young in the tuna stomachs indicates that the reproductive capacity must be very great. The six tuna stomachs that contained the 560 young porcupine fish were taken over a four-day period, and the only tuna that did not contain any was taken on the last day of the tournament. Furthermore, all the small porcupine fish were readily identifiable and none were in an advanced state of digestion. Thus, it is apparent that very large numbers of young porcupine fish were available to the tuna over a period of several days and perhaps for several weeks.

Weights of Viscera

So far as I can determine, there is no published record of the percentages of the total body weight of the bluefin tuna made up by the various viscera. In earlier publications (Krumholz, 1956, 1958) I recorded the relative weights of viscera of eight species of freshwater fishes and those of the Atlantic marlins.

When the fish were opened to examine the gonads and to remove the stomachs and their contents, the heart and all abdominal viscera, with the exception of the kidneys, were excised and weighed. The stomach and intestine were separated from each other and from the caecal mass, slit along their greatest lengths, and any debris and mucous material were rinsed away in sea water. Because of the intertwined arrangement of the caeca within the caecal mass, no attempt was made to remove any materials from the lumina, and the entire mass was weighed in the condition in which it was removed from the fish. The chambers of the heart and the openings of the principal afferent and efferent vessels were washed out with sea water to remove any clotted blood. The connective tissue covering of the spleen was removed before weighing. The gall bladder was carefully separated from the liver in each instance so that the bladder would not become ruptured and the contents lost. The liver required little or no cleaning except for the removal of the ligaments of attachment. Each organ was weighed individually to the nearest gram on a triple-beam balance within a half hour after being removed from the fish. All weights are recorded as wet weights, and from these data the percentage of the total body weight made up by each organ was determined for each fish.

The percentage of the total body weights made up by each organ, and all organs combined, for each of the bluefin tuna examined in this study are listed in Table 2. The body weights and sexes of the individual fish are listed in Table 1. The data on the weights of the organs for tuna No. 1 are omitted because that fish was mutilated by a shark while being caught and the total weight of the fish is not known. For tuna No. 2, only the weight of the heart is included, the other viscera having been discarded inadvertently. From the data at hand it is obvious that the gonads, for each sex, contributed more to the total body weight than any other single organ listed. The percentage of the total body weight contributed by the gonads is followed in decreasing order by the stomach, the caecal mass, the liver, the heart, the spleen, the intestine and the gall bladder. The entire gut, consisting of the stomach, caecal mass and intestine, made up 1.50 percent. of the total body weight, and all viscera combined, 3.57 percent.

If the relative weights of the gonads are not considered, a comparison of the percentage of the total body weight contributed by the viscera of the bluefin tuna with those of seven freshwater fishes, the Atlantic marlins and the sailfish (*Istiophorus americanus*), clearly shows that relatively less of the body weight is contributed by the tuna viscera than those of any of the other fishes listed (Table 3). Among the fishes listed in Table 3, the yellow bullhead (*Ictalurus natalis*), the carp (*Cyprinus carpio*), and the red-

TABLE 2. PERCENTAGE OF TOTAL BODY WEIGHT MADE UP BY EACH ORGAN, AND ALL ORGANS COMBINED, FROM SIX BLUEFIN TUNA, TOGETHER WITH THE AVERAGES, TAKEN NEAR BIMINI, BAHAMAS, MAY 19-23, 1956

Tuna No.	2	3	4	5	6	7	Average
Heart	0.393	0.287	0.337	0.320	0.308	0.330	0.329
Stomach		0.763	0.565	0.745	0.866	0.745	0.737
Caecal mass		0.594	0.594	0.739	0.701	0.686	0.663
Intestine		0.085	0.118	0.096	0.111	0.113	0.105
Liver		0.463	0.648	0.602	0.567	0.628	0.582
Gall bladder		0.016	0.028	0.021	0.046	0.044	0.031
Spleen		0.133	0.140	0.138	0.150	0.119	0.136
Subtotal		2.341	2.430	2.661	2.749	2.665	2.569
Testes		0.993	0.913	1.538	0.627		1.003
Ovaries						1.079	1.079
Total		3.277	3.343	4.188	3.349	3.700	3.571

horse (*Moxostoma erythrurum*) have no caeca in the digestive tract. In the carp and the redhorse there is no good, gross line of demarcation between the stomach and the intestine and, consequently, the entire digestive tube was considered as a single entity.

The data in Table 3 indicate that there are several striking differences in the relative weights of the various organs among the different fishes. The viscera of the yellow bullhead contribute relatively more to the total body weight than in any other species. For that measurement, the yellow bullhead is followed in descending order by the carp, the white marlin (*Makaira albida*), the sailfish, the largemouth bass (*Micropterus salmoides*), the blue marlin (*Makaira ampla*), the bluegill (*Lepomis macrochirus*), the redhorse, the white crappie (*Pomoxis annularis*), the black crappie (*P. nigromaculatus*) and the bluefin tuna. Such an arrangement of fishes bears no relationship to taxonomic order and it is difficult to make any clear-cut, general statement on the basis of food habits. All the species listed are primarily carnivorous, with the exception of the carp and the redhorse (both omnivorous) which are second and eighth, respectively, in the series.

Another striking difference is in the relative sizes of the hearts among the 11 species. Here, the bluefin tuna has a larger heart than any of the others and, on a relative basis, only the hearts of the carp and the sailfish even approach it in size. Nearly all the other species have hearts that are less than half the relative size of the tuna's. It is difficult to propose a theory regarding the size of the heart of the tuna. The heart of a 360-pound blue marlin weighed only 265 grams whereas that of a 361-pound bluefin tuna weighed 524 grams, almost exactly twice as much. Both species are rapid, pelagic swimmers, their principal foods are other fishes (see Krumholz & deSylva, 1958, for foods of marlins near Bimini), and there is considerable overlap in their ranges. However, it is said (Brown 1957, vol. 1:217) that the tuna maintains a body temperature considerably higher (6-12° C.) than the surrounding water, and it may be that the large heart plays an important role in maintaining that higher temperature.

Another comparison between the data for the speared fishes listed here and the bluefin tuna shows that the digestive organs of the white marlin and the sailfish weigh relatively more than twice as much as those of the bluefin tuna, whereas those of the blue marlin are relatively more than 75 percent. heavier. It is possible that such differences in the relative weights of the digestive tracts indicate even greater differences in diet than we may now suspect.

The relative sizes of the livers and gall bladders indicate rather marked differences between the marine and freshwater fishes. Such differences are probably linked with digestive processes.

SUMMARY

The stomach contents of seven bluefin tuna taken near Bimini, Bahamas, in May, 1956, consisted of 560 young-of-the-year porcupine fish, 90 salps, the axial skeletons of 5 small, eel-like fish, 4 protunid crabs, the beak of 1 octopus, and a plant leaf. The gonads of all specimens of tuna appeared to be near spawning condition. The weights of viscera indicated that the relative weight of the heart of the bluefin tuna was nearly twice that of the hearts of each of the Atlantic marlins or the sailfish, and even greater than those of each of seven freshwater fishes.

TABLE 3. AVERAGE PERCENTAGE OF TOTAL BODY WEIGHT MADE UP BY DIFFERENT VISCERA OF THE BLUEFIN TUNA COMPARED WITH THE AVERAGES OF SIMILAR ORGANS FROM SEVEN FRESHWATER FISHES, THE ATLANTIC MARLINS AND THE SAILFISH

	Bluefin Tuna	Blue-gill	Black Crappie	White Crappie	Large-mouth Bass	Yellow Bull-head	Carp	Red-horse	White Marlin	Blue Marlin	Sail-fish
No. of fish	5	11	11	5	5	3	5	8	42	3	2
Heart	0.33	0.16	0.09	0.14	0.08	0.12	0.24	0.13	0.18	0.16	0.20
Stomach	0.74					2.89			1.20	1.26	1.41
		1.32	1.20	1.24	2.40						
Caeca	0.66								2.20	1.18	1.62
Intestine	0.10	0.55	0.39	0.43	0.45	1.76			0.68	0.21	0.67
Entire gut*	1.50	1.87	1.59	1.67	2.85	4.65	1.25	0.99	4.08	2.65	3.70
Liver	0.58	1.19	1.03	1.17	0.84	2.25	3.65	2.00	1.08	0.71	0.72
Gall bladder	0.03	0.21	0.25	0.18	0.26	0.28	0.55	0.27	0.06	0.06	0.06
Spleen	0.14	0.11	0.10	0.06	0.08	0.05	0.13	0.11	0.11	0.09	0.14
Total	2.58	3.54	3.06	3.22	4.11	7.35	5.82	3.50	5.51	3.67	4.82

* The entire gut in this instance consists of the stomach, caeca and intestine. See text for explanation.

However, the total weight of the abdominal viscera and the heart of the tuna was relatively less than similar weights for any other species considered. Also, the digestive organs of each of the marlins and the sailfish weighed relatively more than twice as much as those of the tuna.

LITERATURE CITED

BEEBE, WILLIAM
1936. Food of the Bermuda and West Indian tunas of the genera *Parathunnus* and *Neothunnus*. Zoologica, 21: 195-205.

BIGELOW, HENRY B., & WILLIAM C. SCHROEDER
1953. Fishes of the Gulf of Maine. Fish. Bull. 74, U. S. Dept. Int., Fish and Wildl. Serv., 53: i-viii, 1-577.

BROWN, MARGARET E. (Editor)
1957. The physiology of fishes. New York. Academic Press Inc. Vol. 1, 447 pp.

CRANE, JOCELYN
1936. Notes on the biology and ecology of the giant tuna, *Thunnus thynnus* Linnaeus, observed at Portland, Maine. Zoologica, 21: 207-212.

deSYLVA, DONALD
1956. The food of tunas. Bull. Internat. Oceanogr. Found., 2(1): 37-48.

KRUMHOLZ, LOUIS A.
1956. Observations on the fish population of a lake contaminated by radioactive wastes. Bull. Amer. Mus. Nat. Hist., 110(4): 277-368.
1958. Relative weights of some viscera in the Atlantic marlins. Bull. Amer. Mus. Nat. Hist., 114(5): 402-405.

KRUMHOLZ, LOUIS A., & DONALD P. deSYLVA
1958. Some foods of marlins near Bimini, Bahamas. Bull. Amer. Mus. Nat. Hist., 114(5): 406-411.

NEW YORK ZOOLOGICAL SOCIETY

GENERAL OFFICE
30 East Fortieth Street, New York 16, N. Y.

PUBLICATION OFFICE
The Zoological Park, New York 60, N. Y.

OFFICERS

PRESIDENT	VICE-PRESIDENT	SECRETARY	TREASURER
Fairfield Osborn	Laurance S. Rockefeller	George Wall Merck	David H. McAlpin

SCIENTIFIC STAFF:

John Tee-Van ..... *General Director*
Christopher W. Coates.. *Director, Aquarium*

ZOOLOGICAL PARK

Joseph A. Davis, Jr.. *Assistant Curator, Mammals*
Grace Davall..... *Assistant Curator, Mammals and Birds*
William G. Conway.. *Curator, Birds*
Herndon G. Dowling. *Associate Curator, Reptiles*
Charles P. Gandal .. *Veterinarian*
Lee S. Crandall..... *General Curator Emeritus*
William Beebe..... *Honorary Curator, Birds*

AQUARIUM

James W. Atz..... *Associate Curator*
Carleton Ray..... *Assistant to the Director*
Ross F. Nigrelli..... *Pathologist & Chairman of Department of Marine Biochemistry & Ecology*
Sophie Jakowska..... *Research Associate in Experimental Biology*
C. M. Breder, Jr..... *Research Associate in Ichthyology*
Harry A. Charipper... *Research Associate in Histology*
Homer W. Smith..... *Research Associate in Physiology*

GENERAL

William Bridges .. *Editor & Curator, Publications*
Sam Dunton ..... *Photographer*
Henry M. Lester.. *Photographic Consultant*

DEPARTMENT OF TROPICAL RESEARCH

William Beebe..... *Director Emeritus*
Jocelyn Crane..... *Assistant Director*
David W. Snow ..... *Resident Naturalist*
Henry Fleming ..... *Entomologist*
John Tee-Van ..... *Associate*
William K. Gregory.... *Associate*

AFFILIATES

L. Floyd Clarke ..... *Director, Jackson Hole Biological Research Station*

SCIENTIFIC ADVISORY COUNCIL

A. Raymond Dochez
Alfred E. Emerson
W. A. Hagan
Caryl P. Haskins
John S. Nicholas

EDITORIAL COMMITTEE

Fairfield Osborn, *Chairman*
James W. Atz
William Beebe
William Bridges
Christopher W. Coates
William G. Conway
Lee S. Crandall
John Tee-Van

ZOOLOGICA

SCIENTIFIC CONTRIBUTIONS OF THE NEW YORK ZOOLOGICAL SOCIETY

VOLUME 44 • PART 4 • DECEMBER 31, 1959 • NUMBERS 9 TO 13

PUBLISHED BY THE SOCIETY
The ZOOLOGICAL PARK, *New York*

Contents

9

Heterotopic Thyroid Tissues in Fishes. III. Extrapharyngeal Thyroid Tissue in Montezuma Swordtails, a Guppy and a Cherry Barb[1]

K. France Baker[2]

Department of Zoology, Columbia University, & Genetics Laboratory, New York Zoological Society

(Plate I; Text-figure 1)

HETEROTOPIC (or extrapharyngeal) thyroid tissue in platyfish (*Xiphophorus maculatus*), in the form of thyroid tumors in the kidneys and of normal thyroid tissue in many organs, was recently described (Baker *et al*, 1955; Baker, 1958a, b). Such tissue proved to be common in several inbred laboratory strains of this species. Chavin (1956) reported the frequent occurrence of typical thyroid follicles in the kidneys of normal goldfish (*Carassius auratus*), and occasional heterotopic thyroid tissue has been reported in experimentally-treated guppies (*Lebistes reticulatus*) by Hopper (1952), Vivien & Ruhland-Gaiser (1954) and Pflugfelder (1956). Earlier, displaced thyroid tissues were noted in goitrous specimens of hatchery-raised brook trout *(Salvelinus fontinalis)* by Marine & Lenhart (1911) and Gaylord & Marsh (1912), and in rainbow trout (*Salmo gairdneri)* by Leger (1925). All of the instances of displaced thyroid tissue in guppies and trout cited were regarded as being metastases of pharyngeal thyroid tumors or goiters.

The development of normal and goitrous heterotopic thyroid tissue in laboratory-bred strains of platyfish was shown by Baker (1958b) to be dependent on the concentration of iodine in the aquarium water. Under the lack of iodine, normal heterotopic thyroid tissue appeared early in the lives of these platyfish, and later gave rise to tumors in the kidneys, spleen and other organs. The heterotopic thyroid tissue in platyfish was regarded as derived from the pharyngeal thyroid tissue by a migration of individual cells or follicles, which was stimulated by iodine deficiency.

Iodine level was also shown to be extremely important in the development of pharyngeal goiter in laboratory-raised Montezuma swordtails (*Xiphophorus montezumae*) by Berg, Gordon & Gorbman (1954). Pharyngeal thyroid tumors in these fish were first reported by Gorbman & Gordon (1951), and it was found that up to 100% of the Montezumas developed thyroid tumors by the age of 6-12 months (Berg, Edgar & Gordon, 1953). While these observations were being made, the swordtails in question were living in the same laboratory environment as the platyfish which later were found to produce heterotopic thyroid tumors (Baker *et al*, 1955; Baker, 1958a).

It thus became of interest to discover whether thyroid tissue in Montezuma swordtails was as widely spread in the body as in the platyfish studied, and whether heterotopic thyroid tissue, if present, formed extrapharyngeal thyroid tumors in concert with the pharyngeal thyroid tumors. If tumors of renal thyroid were formed, their development was necessarily less than in the platyfish, as these tumors in platyfish caused a tremendous swelling of the body and this symp-

[1]Part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University. This work was supported by a research grant (C-297) to Dr. Myron Gordon of the New York Zoological Society from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and by a predoctoral research fellowship (CF-6184) from the National Cancer Institute.

[2]Present address: Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York 32, New York.

tom was not commonly seen among the swordtails.

The question also was raised as to whether heterotopic thyroid tissue was commonly to be found in fish, especially normal freshwater teleosts living under conditions were iodine was deficient. With this in mind, individual specimens of the guppy and the cherry barb (*Barbus titteya*) which were brought to our attention received careful examination and are described in this report. A specimen of *Xiphophorus xiphidium* which was afflicted with both a thyroid tumor in the kidneys and a melanoma will be described separately (Gordon, Alexander and Baker, unpublished).

Materials and Methods

1. *Fish Studied and Laboratory Conditions.*—Twenty-one adult Montezuma swordtails were examined in this study. Six of these fish were swollen and were examined for renal thyroid tumors. Their age was not known, but it was in excess of a year. The remaining 15 fish were normal in appearance and were selected at random from the breeding colony to be examined for heterotopic thyroid. Their ages ranged from 9 to approximately 33 months.

These Montezuma swordtails were all descendants of wild fish captured in the Rio Axtla, Mexico, in 1939, by Dr. Myron Gordon (Gorbman & Gordon, 1951). The wild fish were closely inbred in the laboratory and the original stock was divided into several sublines.

The Montezuma swordtails were maintained in the laboratory side by side with strains of platyfish which developed large amounts of extrapharyngeal thyroid tissue. Several analyses made of the laboratory aquarium water showed that it contained much less iodine (0.5 μg/1—Berg, Gordon & Gorbman, 1954; 0.8 and 1.0 μg/1—Baker, 1958a) than a water sample taken from the Rio Axtla (3.3 μg/1—Berg, Gordon & Gorbman, 1954). New York City tap water contained 2.3 μg/1 of iodine (Berg, Gordon & Gorbman, 1954) and the laboratory water was believed to have been depleted of iodine from its original state by reuse and the turnover of plants, snails and fish for a period of several years (Berg, Gordon & Gorbman, 1954).

The guppy described here was a mature pregnant female of unknown age, which had been used in an experiment in the Department of Fishes of the American Museum of Natural History. The conditions under which it had lived are unknown. It was obtained in the form of a completed serial section from Miss Priscilla Rasquin, of the Department of Fishes.

The cherry barb came from a home aquarium, whose water was not analyzed. The fish was of unknown age and its sex was not determined.

2. *Histological Methods and Radioautography.*—All fish examined (except the guppy) were fixed in Bouin's fluid, containing formic acid as a decalcifier, embedded in paraffin and sectioned serially at 10 μ. The sections were stained for histological examination with hematoxylin-eosin.

Radioautographs, using tracer doses of I^{131} for the detection of thyroid tissue, were carried out as previously described (Baker, 1958a). Eleven of the 21 Montezuma swordtails studied were radioautographed.

Results

1. *Montezuma Swordtails.*—Only one of the six swollen Montezumas proved to have a thyroid tumor in its kidneys, although such tumors were the common cause of similar symptoms in platyfish (Baker *et al,* 1955). The enlarged kidneys found in the remaining five fish resulted from glomerular cysts; no thyroid tissue occurred in the kidneys of three of them (Table 1). In the two which had some thyroid tissue in their kidneys, this consisted of scattered follicles which by no means could be classed as a tumor. Two of the five fish with cystic glomeruli were radioautographed with I^{131}: one had renal thyroid tissue, the other lacked it.

Nine of the 15 normal Montezumas examined were radioautographed. Although 14 of these fish had pharyngeal thyroid tumors, ranging in development from loose hyperplasia to very large dense growths with gill invasion, only five had thyroid tissue in the kidneys (Table 1). In no case could this tissue be regarded as a tumor. Although the renal thyroid cells were hypertrophied and colloid was sparse, the cells formed only scattered individual follicles or small clusters of follicles, never large masses. These features made this thyroid tissue resemble that found in the hearts of platyfish which had thyroid tumors in their kidneys (Baker *et al,* 1955).

Heterotopic thyroid tissue in organs other than the kidneys seemed to be rare in Montezuma swordtails. None was found in the heart of any of the radioautographed fish—in which it would most readily have been noted. The chorioid glands of six fish were examined, but only one had thyroid tissue in that organ. This fish was a pregnant female which also had renal thyroid follicles. The spleen of none of these fish was examined.

The thyroids of embryos contained in the pregnant female autographed strongly, but no

Table 1. Incidence of Renal Thyroid and Its Degree of Development, as Compared with Pharyngeal Thyroid, in Inbred Montezuma Swordtails

A. Fish with enlarged kidneys[1]

Subline	Total Examined	Number with Renal Thyroid	Degree of Renal Thyroid Development	Degree of Pharyngeal Thyroid Development
35	1	0	0	Slight hyperplasia
38	1	0	0	Thyroid tumor
43	3	2	Scattered follicles	Hyperplastic
Unknown	1	1	Thyroid tumor	Large thyroid tumor

B. Fish appearing normal

Subline	Total Examined	Average Age (months)	Number with Renal Thyroid	Degree of Renal Thyroid Development	Degree of Pharyngeal Thyroid Development
35	11	12.5	2	Scattered, hypertrophied follicles	Small to medium-sized tumors—1 normal
38	4	32	3	Small thyroid masses	Large thyroid tumors

[1]The swollen kidneys in the five fish which had little or no thyroid tissue in their kidneys proved to be the result of cystic glomeruli.

other parts of the embryos or yolk did so. This was similar to the results obtained on embryonic platyfish (Baker, 1958a).

In summary, only one Montezuma swordtail was found to have a true tumor of thyroid tissue in the kidneys. This tumor was similar to those found in the kidneys of platyfish. Only 35% of the adult Montezumas had thyroid tissue in their kidneys, in contrast to 94% of the normal *BH* platyfish of the same ages living under similar conditions. No significant difference was apparent between different sublines of Montezuma swordtails, with respect to the development of heterotopic thyroid tissue. Those differences seen in Table I seem related only to age (see Baker, 1958a).

2. *Guppy*.—The serial sections of this fish were given to the author because it was known that thyroid follicles were present in the spleen. Further examination of the slides revealed no thyroid tissue in the kidneys or heart. The eyes had been removed, so that the chorioid gland could not be examined. Pharyngeal thyroid follicles were numerous, but quiescent (Pl. I, fig. 1), and no follicles occurred in the gills. In the spleen the follicles were very numerous and they resembled the ones in the pharyngeal area (Pl. I, fig. 2). Close to the spleen, among the pancreatic tissue, thyroid follicles also occurred. Some of these were tubular in shape, but their epithelium and colloid was like that of the follicles in the spleen and pharynx. The occurrence of thyroid tissue in the spleen without the concurrent occupation of the kidneys by this tissue was strikingly different from the distribution of the heterotopic thyroid tissue found in platyfish (Baker, 1958a).

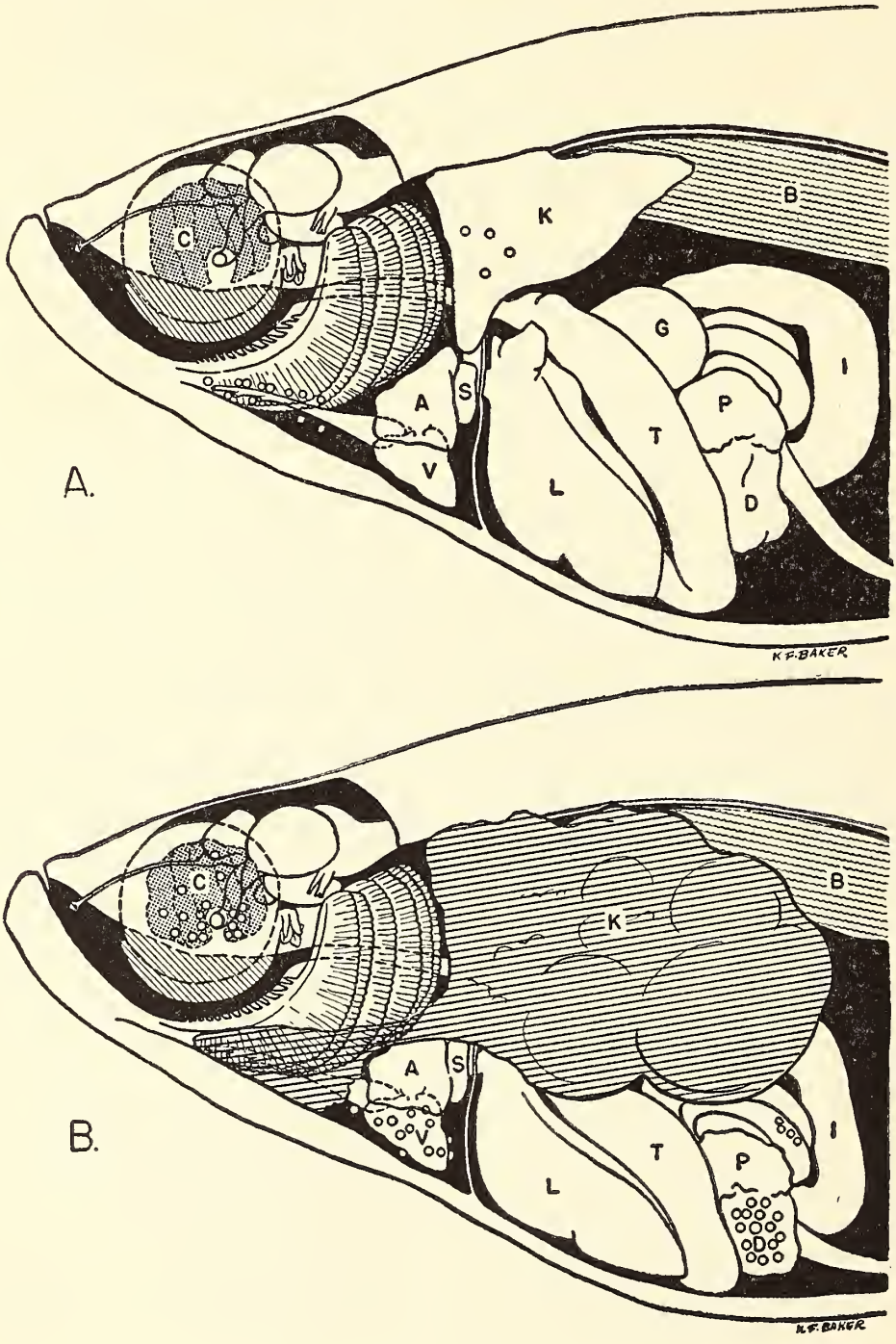
3. *Cherry Barb*.—This fish's body was very swollen, but it had no externally visible enlargement of the pharyngeal thyroid. Serial sections revealed tumorous thyroid tissue in both pharynx and kidneys. The pharyngeal tumor was made up of a large solid mass of both follicular and afollicular tissue (Pl. I, fig. 3). The follicles, although their cells were much hypertrophied, contained large amounts of colloid, which was extensively vacuolated at the cell surface. The nuclei of the cells were distal to the colloid, and were more basophilic and smaller than the nuclei of the thyroid tumor cells in platyfish (Baker *et al*, 1955). Thyroid follicles were numerous in the gills, appearing in chains along the branchial blood vessels (Pl. I, fig. 4). Thyroid follicles were also numerous in the connective tissue above the pseudobranch and pharynx, and on the surface of blood vessels entering the pseudobranch. In the pericardial cavity, thyroid follicles occurred in groups around the bulbus arteriosus and on the surface of the ventricle. No thyroid tissue was found inside the heart or in the eye.

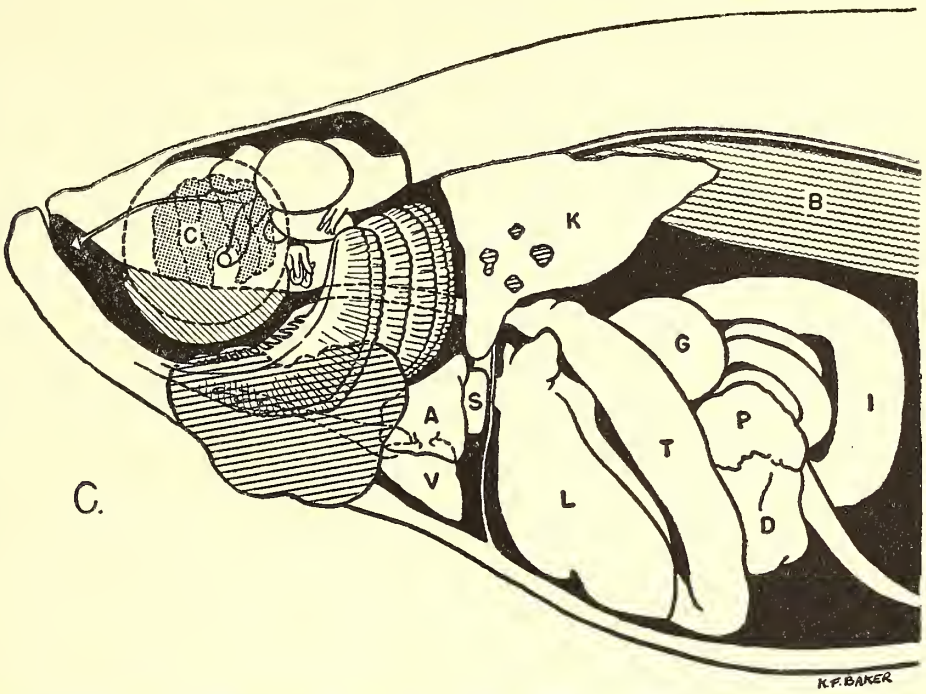
The kidney tumor of the cherry barb, as in the platyfish renal thyroid tumors, formed large cysts (Pl. I, fig. 6), which were the chief cause of the abdominal distension. In the cherry

barb the kidney tumor consisted chiefly of colloid-laden follicles, resembling those in its pharyngeal area (Pl. I, figs. 5, 6), but in the platyfish the tumors were largely without colloid. In the barb, thyroid tissue also occurred in the mesenteries and connective tissue around the gut, a location never found to be occupied by thyroid tissue in platyfish.

Discussion

The results reported here support the hypothesis that in fish, or at least in freshwater teleosts,





TEXT—FIG. 1. Variation in Thyroidal Development in Xiphophorin Fishes. Normal thyroid tissue is represented by open circles. Goitrous thyroid tissue is represented by diagonally shaded masses.

A. Platyfish of strain *30*. This strain has never been observed to develop any type of thyroid tumor.

B. Platyfish of strain *BH*. A high frequency of thyroid tumors involving the kidneys, and to a lesser extent, the pharyngeal thyroid tissue, occurred in this strain.

C. Montezuma swordtail. A very high frequency of pharyngeal thyroid tumors occurred in this species. Although thyroid tissue was found in the kidneys of some of these fish, a thyroid tumor in the kidneys like those found in the strain *BH* platyfish was found only once among the stock.

KEY: **C**, chorioid gland; **A**, auricle; **V**, ventricle; **S**, sinus venosus; **K**, kidney; **L**, liver; **T**, stomach; **G**, gall bladder; **P**, pancreas; **D**, spleen; **I**, intestine **B**, air bladder.

thyroid tissue may often be found in areas other than the pharyngeal one. To date, such tissue has been found in specimens of eight species of freshwater fish, representing six genera. All of these were fish raised in captivity, and the specimens of the three species of *Xiphophorus* were raised in water known to have a very low iodine content, compared with that of native waters. Indirect evidence indicates that extrapharyngeal thyroid tissue may occur in wild specimens of a species of freshwater teleost native to the American "goiter belt," the Great Lakes region. This inference was made by Baker (1958a) from a peculiarity in the iodine metabolism of *Fundulus diaphanus*, as reported by Gorbman & Berg (1955), and it receives some support from reports of the common occurrence of pharyngeal goiter in wild fish from the Great Lakes (Marine & Lenhart, 1910). We conclude that scarcity of iodine may lead to the appearance of heterotopic thyroid tissue in freshwater teleosts, in either the domesticated or wild state.

The results set forth here, however, as well as those previously described by Baker (1958a), show that great differences in the response of thyroid tissue to environments that are very similar with respect to iodine and other known factors may occur between different species of fish, or between different strains of a single species. Among strains of platyfish, for example, the *30* strain never produced any thyroid tumors, either pharyngeal or heterotopic, although normal renal thyroid tissue was found in 36% of the adult fish examined. A closely related offshoot from the same wild-caught ancestors, strain *163*, did produce thyroid tumors in the kidneys. Two long-domesticated strains of platyfish, *BH* and *Fu*, produced an abundance of very large thyroid tumors in the kidneys, and all *BH* strain fish had thyroid tissue in their kidneys by the age of one

year. None of the platyfish strains produced large thyroid tumors in the throat, regardless of the state of their kidneys. On the other hand, Montezuma swordtails proved to have a high incidence of very large pharyngeal thyroid tumors, but very little extrapharyngeal thyroid tissue; that is, about the same amount as in the strain *30* platyfish.

Thus, three variations of thyroidal development have been found in xiphophorin fishes living in an environment poor in iodine (Text-fig. 1): (1) heterotopic thyroid, but no tumors present; (2) tumors of both pharyngeal and heterotopic thyroid present; and (3) tumors of pharyngeal thyroid present but without heterotopic thyroid becoming hyperplastic. The development of tumors in the pharynx and other locations was never found to be equal. In platyfish, thyroid kidney tumors became large and lethal, while the thyroid in the throat was only moderately goitrous; in the Montezumas, the pharyngeal tumors became massive, obstructing the gills, while thyroid tissue in the kidneys was scarcely noticeable.

As all these xiphophorin fish stocks were raised in the same laboratory, sharing a more or less common pool of tank water, plants and snails, and were fed the same diet and subjected to the same conditions of temperature and light, it is assumed that the only consistent source of group differences must be genetic. This was borne out by the persistence of the characteristic group patterns for many generations.

An attempt was made to analyze genetic factors responsible for the differences between the thyroidal responses of the *30* and *BH* strains of platyfish. F_1 and F_2 hybrids between these strains were raised and the incidence of renal thyroid tumor in each generation was compared with that of the parent stocks. The results supported a hypothesis that several genes were involved in the production of the tumors (Baker, 1958a).

The limited data on Montezuma swordtails presented here give no indication that genetic differences occur between strains in the production of heterotopic thyroid tissue. Strain differences were found, however, in pharyngeal thyroid tumor development in these fish by Berg, Edgar & Gordon (1953). It was suggested that increasing homozygosity of multiple factors was responsible for enhanced thyroid tumor development in the most inbred strain. The strains of Montezuma swordtails have not been subjected to further genetical analysis for factors affecting thyroid development.

Summary

1. Adult Montezuma swordtails of several inbred laboratory strains were examined for the presence of heterotopic thyroid tissue. Although all these fish had pharyngeal thyroid tumors, only 35% of them had any thyroid tissue in the kidneys, and this tissue was never abundant. Thyroid tissue was rarely found in heterotopic locations other than the kidneys.

2. Only one Montezuma swordtail was ever found to have a thyroid tumor in its kidneys.

3. The Montezumas may be contrasted with the *BH* strain of platyfish, in which 100% of the fish had thyroid in their kidneys by the age of a year, and in which this tissue frequently developed into large tumors. Both the Montezumas and *BH* platyfish lived in similar environments in which iodine was known to be in low concentration.

4. Heterotopic thyroid tissue, in normal and tumorous conditions, is described in specimens of the guppy and the cherry barb.

5. It is suggested that heterotopic thyroid tissue may be common in freshwater teleosts living in iodine-poor environments.

Acknowledgments

I wish to express my gratitude to Prof. L. G. Barth and Prof. Aubrey Gorbman of Columbia University, and to the late Dr. Myron Gordon of the New York Zoological Society, for their encouragement and helpful discussion throughout these studies, and particularly to Dr. Gordon for the use of the facilities of the Genetics Laboratory of the New York Zoological Society, including its inbred strains of xiphophorin fishes. I want to thank Miss Priscilla Rasquin of the Department of Fishes of the American Museum of Natural History for the gift of the slides of the guppy discussed here, and also to express my appreciation to Dr. Olga Berg for her preparations of the Montezuma swordtail which exhibited a renal thyroid tumor.

Addendum

While this paper was in proof, Dr. Madeleine Olivereau reported (personal communication) the occurrence of thyroid follicles in the kidneys of an individual of *Typhlogarra widdowsoni*, a blind cave cyprinid fish from Iraq. In this fish, which proved to have pharyngeal thyroid "adenoma," both hypertrophied and normal thyroid follicles occurred in the kidneys. In this it resembled the cherry barb described in this paper. The *Typhlogarra* had been maintained for three years in the laboratory, under light and temperature conditions simulating those of the environment of original capture. Other blind cave fish, from Ethiopia, kept under the same conditions for two years, failed to exhibit thy-

roid hypertrophy. I believe it remains possible that the *Typhlogarra* was more sensitive to lowered iodine intake, or that its normal requirement for iodine exceeded that of the other cave fish.

Dr. Olivereau's observations will be published in the near future.

Literature Cited

Baker, K. F.

1958a. Heterotopic thyroid tissues in fishes. I. The origin and development of heterotopic thyroid tissue in platyfish. J. Morph., 103: 91-133.

1958b. Heterotopic thyroid tissues in fishes. II. The effect of iodine and thiourea upon the development of heterotopic thyroid tissue in platyfish. J. Exp. Zool., 138: 329-353.

Baker, K. F., O. Berg, A. Gorbman, R. F. Nigrelli & M. Gordon

1955. Functional thyroid tumors in the kidneys of platyfish. Cancer Res., 15: 118-123.

Berg, O., M. Edgar & M. Gordon

1953. Progressive growth stages in the development of spontaneous thyroid tumors in inbred swordtails. Cancer Res., 13: 1-8.

Berg, O., M. Gordon & A. Gorbman

1954. Comparative effects of thyroidal stimulants and inhibitors of normal and tumorous thyroids in xiphophorin fishes. Cancer Res., 14: 527-533.

Chavin, W.

1956. Thyroid distribution and function in the goldfish, *Carassius auratus* L. J. Exp. Zool., 133: 259-279.

Gaylord, H. R. & M. C. Marsh

1912. Carcinoma of the thyroid in the salmonid fishes. Bull. Bur. Fish., 32: 363-524.

Gorbman, A. & O. Berg

1955. Thyroidal function in the fishes *Fundulus heteroclitus, F. majalis,* and *F. diaphanus.* Endocrinol., 56: 86-92.

Gorbman, A. & M. Gordon

1951. Spontaneous thyroidal tumors in the swordtail *Xiphophorus montezumae.* Cancer Res., 11: 184-187.

Hopper, A. F.

1952. Growth and maturation response of *Lebistes reticulatus* to treatment with mammalian thyroid powder and thiouracil. J. Exp. Zool., 119: 205-217.

Leger, L.

1925. Tumeurs observées chez les Salmonides d'élévage. Compt. rend. Assoc. franc. p. Advance. Sci., 49: 395-396.

Marine, D. & C. H. Lenhart

1910. On the occurrence of goitre (active thyroid hyperplasia) in fish. Bull. Johns Hopkins Hosp., 21: 95-98.

1911. Further observations and experiments on the so-called thyroid carcinoma of brook trout and its relation to endemic goitre. J. Exp. Med., 13: 455-475.

Pflugfelder, O.

1956. Wirkungen von Epiphysan und Thyroxin auf die Schilddrüse Epiphysektomierter *Lebistes reticulatus* Peters. Roux' Archiv. f. Entwicklungsmekanik, 148: 463-473.

Vivien, J. & M. Ruhland-Gaiser

1954. Étude préliminaire de goitres envahissants spontanés, rencontres chez un cyprinodonte: *Lebistes reticulatus r.* Ann. Endocrinol., 15: 585-594.

EXPLANATION OF THE PLATE

Plate I

Fig. 1. Pharyngeal area of a guppy, showing normal thyroid follicles around the ventral aorta. × 140.

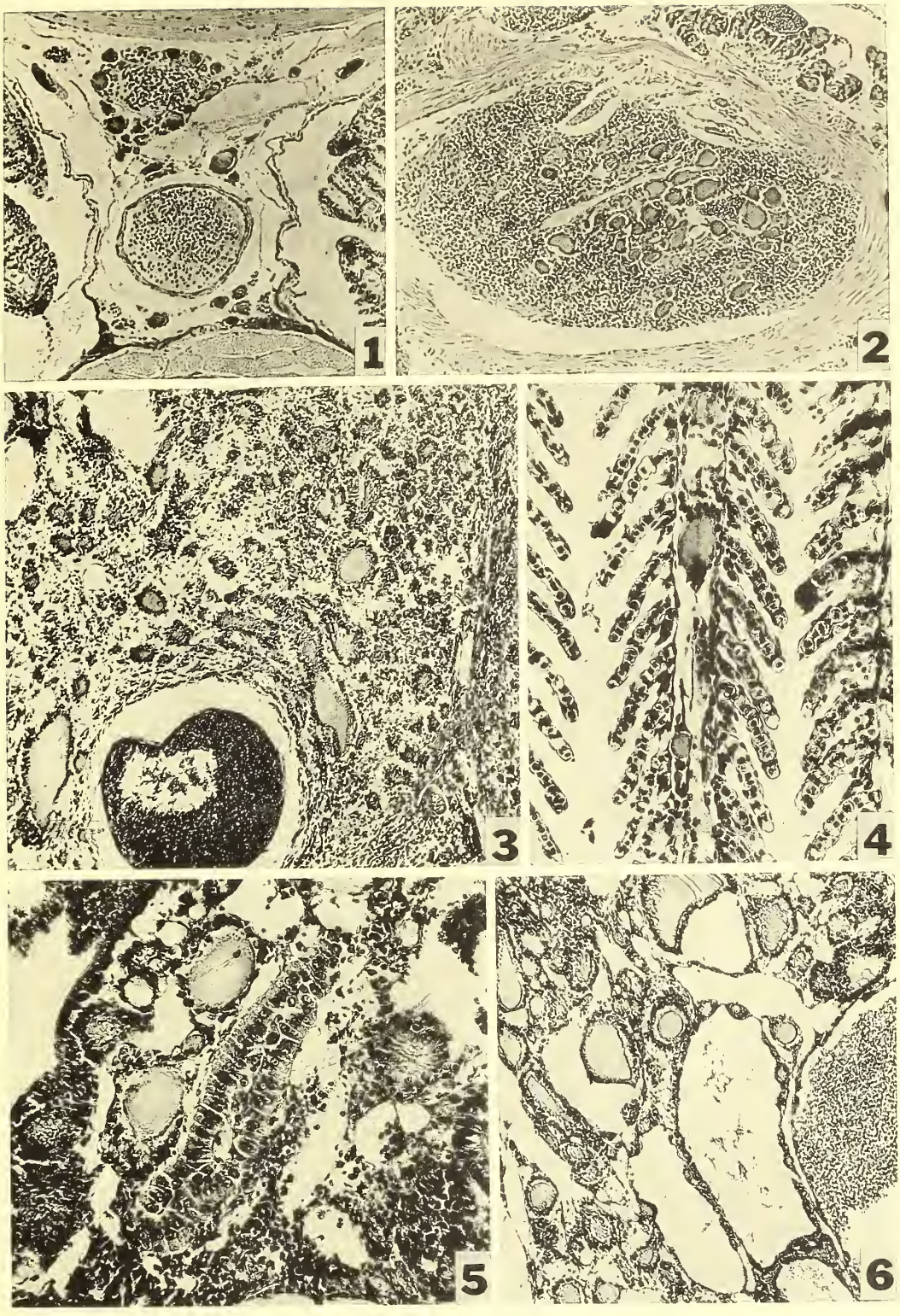
Fig. 2. Thyroid follicles in the spleen of the same guppy. These follicles are morphologically similar to those in the pharyngeal area. × 140.

Fig. 3. Thyroid tumor in the pharyngeal area of a cherry barb. The ventral aorta is at the bottom left. × 130.

Fig. 4. Thyroid follicles along the branchial blood vessels of the same fish. None were found to be inside these vessels. × 300.

Fig. 5. Thyroid tumor in the kidney of the same cherry barb, showing a kidney tubule surrounded by thyroid follicles and cells. × 280.

Fig. 6. Thyroid follicles and cysts composing the thyroid tumor in the kidney of the cherry barb. No kidney tissue is visible in this photograph. × 130.



EXTRAPHARYNGEAL THYROID TISSUE IN MONTEZUMA SWORDTAILS,
A GUPPY AND A CHERRY BARB

10

Observations on the Spawning Behavior and Egg Development of *Strongylura notata* (Poey)

C. M. Breder, Jr.

The American Museum of Natural History

(Plates I & II; Text-figure 1)

Introduction

During extended field work in southern Florida and the Bahamas, from 1928 to 1959 the very common needlefish, *Strongylura notata* (Poey), managed to reproduce close to where our work was being carried out, without yielding easy clues as to the details of this process. Not until 1941 were eggs successfully obtained by stripping[1] and not until 1959 were observations made on reproductive behavior. The places where attempts were made to further these studies include the Florida locations of Key West, the Dry Tortugas, Palmetto Key in Pine Island Sound, Cape Haze on Gasparilla Sound and Manasota Key on Lemon Bay, and the Bahama locations of Nassau, the Berry Islands, Andros Island and Bimini. A preliminary report on the earlier work, Breder (1932), includes data to that date. The present report is released at this time as it is now possible to outline what are evidently the basic features of both egg development and the breeding behavior together with various ecological items and suggestions. It is believed that this may interest and encourage others to notice features of the activities of these fishes that might otherwise be easily missed or ignored. The account of the development of the eggs is based on work done at the Palmetto Key Laboratory of the New York Aquarium. That of the spawning behavior is based on observations made in Lemon Bay from the author's property while working out of the Cape Haze Laboratory.

[1] An amusing account of some of the tribulations incident to the study of these eggs has been given by Bridges (1942).

Spawning Behavior

Although it is not particularly difficult to obtain hatchable eggs from *Strongylura notata* (Poey), by stripping and artificial fertilization, the eggs have not been described, nor has the reproductive behavior. It has long been suspected by the author that these fish spawned in the tangles made by the looping roots of red mangrove, *Rhizophora mangel.* Such a situation practically precludes direct observation. Approach by skiff, close enough to peer into the tangle, scatters the fishes. This is not a matter of sound of oars for it is possible slowly to approach such situations in almost complete silence by drifting in from the proper direction with respect to tidal flow. It is evidently the nearness of the mass of the hull that induces the scattering, principally on an optical basis. A land approach is even worse in this respect as it is impossible to make one's way through the mangroves without considerable commotion. A view from above is precluded by the heavy overhanging foliage. In many places in southern Florida and the Bahamas, it was noted that reproductively-ready fishes would be found in small groups near large mangrove growths always on the lee side and moving in and out of the shelter of the dangling aerial roots.

It was not until a special circumstance arose that the further likelihood of this site of spawning was established and that the actual spawning behavior was observed. In a particular situation on Lemon Bay, Florida, the red mangroves had been stripped off, leaving the black mangrove, *Avicennia nitida,* as the most seaward tree. Here the water surface was fully exposed

for observation to the now sandy shore where the water lapped at the pneumatophores. This condition was further enhanced by the incidental presence of a dock running out from shore for nearly a hundred feet. After a rather heavy north wind in mid-April the shore was floored with a deep bedding of samples of the softer algae of the Bay, consisting largely of *Gracilaria.* When the wind veered around to the southwest and became light, on April 19, *Strongylura* began to appear in small bands and hung over the algae beds in the semi-shelter of the overhanging branches of the single black mangrove tree at that point, which is shown in Plate I, fig. 1, as viewed from the dock. By 11:00 A.M. on April 20 a considerable congress had formed at this point. About a dozen of the smaller-sized fishes, believed to be males, hung in a loose aggregation. Occasionally one or sometimes two, larger, fatter fish, believed to be females, would come in from further offshore, swimming usually in a direct line to the aggregation, as though they were certainly directed to it. They would join the group, which would then mill about more actively. See Plate I, fig. 2. One or more of the smaller fish would come up from behind to lie parallel with the larger fish. This action caused an increased milling about, as the fish jockeyed for position. The larger fish would then swim away from the shore, roughly at right angles to it and usually further than the end of the dock, with a varied number of the smaller fish following. These tended to trail behind, sometimes making a troupe of as many as ten, which on occasion fell into single file, although usually the formation was not as regular as that. See Plate II, fig. 3. The remaining aggregation then slowly grew to its former size by the gradual accretion of individual fishes of the smaller sizes which joined the group. It is not clear whether these were the same fish returning or simply new recruits. Later larger fish would join and the whole performance would be repeated. It is practically certain that at least two of the larger-sized fishes returned repeatedly. It is doubted that there was any spawning, but it does seem that this was some kind of pre-spawning maneuvering. It may be that the swimming out to open water by a female served as a cue to the males that she was not quite ready for spawning.

This behavior continued intermittently throughout most of the day. High tide occurred at 1:03 P.M. and low at 7:17 P.M. On the falling tide at 4:00 P.M. spawning or spawning attempts were in progress. Two fish would run side by side between two clumps of red algae and take a momentary stance similar to that taken by *Fundulus,* insofar as their elongate and rather stiff bodies permitted, and "shiver" in appropriate fashion. Before and after such performances, others caused such turmoil by similar but more vigorous activity that the details could not be seen. In all cases of this activity, the fish faced toward shore in three to six inches of water. At no time was there any evidence of wrestling between the males as is done by the related hemiramphid, *Dermogenys,* the nearest relative on which there is any behavior data. The spawning activity continued until darkness at which time all the fish departed. The entire activity occurred in a place that would have been completely hidden from sight if the *Rhizophora* had been left standing.

Wandering bands of *Mugil cephalus* Linnaeus passed nearby these spawning *Strongylura* without heeding them, but after the activity had reached its maximum, the *Mugil* closed in under the spawners and actively fed on something under them, evidently eggs. See Plate II, fig. 4. This went on for some time, not in the least interfering with the *Strongylura* which, naturally, were just under the water's surface most of the time. This area was carefully searched for eggs and later when low tide exposed the place another search was made but without success. Since the eggs are nearly 4 mm. in diameter they should not have been very hard to find if present in any considerable numbers. Since these observations were made at the very onset of the general spring increase in spawning activity, as measured by the nearly total absence of postlarval *Strongylura* from sight observations and from townet material, presumably the egg production was little and probably mostly fell to such fishes as *Mugil.* Two days later the wind swung slowly to the north and in so doing whipped the beach clean of the loose algae. No further activity was seen at that place for the rest of the stay, which lasted for nearly another month. However, observations at a small prominence in Lemon Bay a little north of the site described and of such a nature that it presented *Rhizophora* growth facing in nearly every compass direction, showed that there were similar *Strongylura* gatherings in and about those which happened to be showing a lee exposure and that there were none at places facing into the wind. As noted previously, it was impossible to see just what did transpire in these places. Here again it was impossible to find eggs, although a considerable amount of soft growths, brush and similar things were examined.

Egg Development

The egg development is exceedingly slow,

which is in keeping with that of the only other species in the family of which the incubating period is known. Dantan (1905) reported 35 to 36 days to hatching time for Mediterranean *Belone belone* (Linnaeus). Ryder (1882) was only able to carry the eggs of *Strongylura marina* (Walbaum) to nearly seven days, well short of full development. These averaged 3.6 mm. in diameter. Raffaele (1895), Ehrenbaum (1897) and Borcea (1933) all describe the eggs of *Belone,* which are evidently a little less than 2 mm. in diameter. Table 1 and Text-figure 1 indicate the time in days at which various embryological features of *S. notata* appeared. The eggs averaged a little less than 4 mm. in diameter and mean of six 3.95+ and ranged from 3.67— to 4.18—, sank in sea water and possessed rather uniformly distributed adhesive threads which held them in bunches. See Plate II, fig. 5. Eggs taken near Key West, Breder (1932), were smaller, ranging from 3.20 to 3.30 with mean at 3.25 mm. It was impossible to fertilize them because of the absence of ripe males.

The present eggs, the product of a single ripe female seined with a group of males and unripe females on the flats west of Useppa Island in Pine Island Sound late in the afternoon, were fertilized at the Palmetto Key Laboratory at 5:05 P.M. July 5, 1941. The eggs were notably amber-colored. This darkened with the passage of time, which made details of the contents increasingly difficult to discern. It was possible to carry these eggs into the tenth day. Because of practical difficulties it was impossible to keep the temperature as well regulated as desirable in the later portion of the incubation, as may be seen by the temperatures in Table 1. Whether or not this difficulty was responsible for the failure of the eggs to go on to hatching is not known. The experience of Ryder (1882) was rather similar and he presumably supplied his eggs with running sea water or at least had the container in a bath of running sea water.

Most of the details of development are evident from the drawings and Table 1, obviating a lengthy description of the embryological events. It should be noted, however, that only xanthophores were developed, although the embryo had well-developed fin rays and the beginnings of the beak were evident. The reduction of the yolk along with these features of development indicated, however, that the time of hatching was not far distant. Table 2 gives figures of the greatest diameter of the yolk, measured at intervals during the development and an estimation of the volume. As the yolk is normally consumed at an accelerating pace as the embryo grows larger, passing in this instance from a mean of 0.14 mm.^{3} between the first two measurements to one of 1.56 mm.^{3} between the last two, this also suggests the rapid approach of hatching. Also the beak of the smallest certainly identifiable individual of this species, as measured by Breder (1932), had a standard length of 14 mm. and an upper jaw of 1.6 mm. Making measurements in the same way of the drawing in Text-figure 1 F, according to the diameter of the yolk and measuring to the base of the tail, a standard length estimate of 11.6 is obtained with the upper jaw measuring 0.38 mm. Thus in this embryo the beak is 0.03% of the standard length while that of the smallest fish is 0.11%. These various measures taken together suggest that these eggs would hatch, under the specified conditions, in a little more than two weeks.

Discussion

A comparison of the development of the eggs of *Strongylura notata* with that of its more northerly-ranging congener *S. marina* is possible because of the studies of Ryder (1882). The embryologic development is naturally very similar, but the developmental pace is much faster in the former, as is indicated in Table 3. From this it is clear that *S. notata* is developing over twice as fast as *S. marina,* it taking the former only 45.9% of the time it took the latter to reach stage "F."The single negative value in the "Difference" column is accounted for by difficulties in estimating equivalent stages of development and probably does not represent a change in the pace of development. This case, stage "B," the blastodisc, shows little that can serve as a landmark for a comparatively long time. As can be seen from Table 1, the *S. notata* eggs were developing in temperatures not very dissimilar to those in bays where they live, as is indicated by the column of temperatures marked "At dock" compared with those where the eggs were incubating marked "In bowl." Ryder's temperatures, although not given in his paper, were probably at least as close to the Woods Hole water temperatures, very considerably less than those to be expected in the shallow bays of the Florida Gulf Coast. Water temperature records at Woods Hole show that it seldom exceeds 70°, and then only a fraction of a degree, while during the summer the temperatures are mostly in the middle or upper sixties. Compared with this, the temperatures at the laboratory dock side in Pine Island Sound ranged from 73.1 to 80.6. It thus may be that the differences in developmental rates are mostly the result of differences in temperature.

TABLE 1. PROTOCOL OF THE DEVELOPMENT OF EGGS OF *Strongylura notata* (POEY) ARTIFICIALLY FERTILIZED AT THE PALMETTO KEY LABORATORY, JULY, 1941

Hour	Water Temperature		Rate per Minute		Development and Remarks
	At Dock	In Bowl	Heart-beat	Respi-ration	
			July 5		
5.05 p.m.	80.6	79.2			Fertilized.
7:25 p.m.					8 cell stage. Text-fig. 1, A.
7:45 p.m.					16 cell stage.
9:00 p.m.	79.1	75.2			Cells no longer countable.
			July 6		
7:50 a.m.					Germinal disc evident. Text-fig. 1, B.
1:30 p.m.		79.1			G. disc about ¼ down, primitive streak.
5:15 p.m.					Somites and Kupfer's vesicle evident.
8:15 p.m.	73.1	79.1			Blastopore closing. Text-fig. 1, C.
			July 7		
8:00 a.m.		74.2			Eyes forming. Text-fig. 1, D.
10:00 a.m.			102.5		Heartbeat evident.
1:20 p.m.			113.2		Xanthophores appearing.
7:00 p.m.					A few blood cells in circulation.
8:30 p.m.					Slight movements. Plate II.
			July 8		
11:00 a.m.	79.1	74.1	150.0		Xanthophores increasing.
2:30 p.m.		81.2	157.0		Embryo darkening, blood showing many cells. Text-fig. 1, E.
5:00 p.m.		80.1	176.5		Pectorals evident, slight "beak" evident.
8:00 p.m.		78.6	166.7		One egg hatched prematurely.
			July 9		
10:00 a.m.	73.1	77.2	169.4		Pectorals waved, opacity of shell increasing.
6:00 p.m.		80.1	176.5		Tail spatulate, xanthophores more intense yellow.
9:20 p.m.		77.7	153.4		Pectorals waved almost continuously.
			July 10		
7:30 a.m.		75.6	157.0		Pectorals with regular rhythmic movements.
			July 11		
7:20 a.m.		79.2	162.2	34.2	Respiration begun, yolk diameter 2.7 mm.
6:30 p.m.		74.1	142.8	55.0	Eyes darkening.
			July 12		
7:25 a.m.		75.2	133.2	25.4	Iris silvery, fin rays in tail, blood markedly red.

TABLE 1. PROTOCOL OF THE DEVELOPMENT OF EGGS OF *Strongylura notata* (POEY) ARTIFICIALLY FERTILIZED AT THE PALMETTO KEY LABORATORY, JULY, 1941 *(Continued)*

Hour	Water Temperature		Rate per Minute		Development and Remarks
	At Dock	In Bowl	Heart-beat	Respi-ration	
7:45 a.m.		77.1	146.2	75.0	Tail just reaches tip of snout.
			July 13		
7:20 a.m.		77.2	133.2	77.4	Mandibular valves evident and working.
11:15 p.m.		73.1	122.1	53.9	Anal and dorsal rays well developed.
			July 14		
8:45 a.m.		70.1			Diameter of yolk 2.4 mm.
10:30 a.m.			118.0	48.4	
12:20 p.m.		73.5			Beak forming. Text-fig. 1, F. Ribs and neural spines evident.
5:00 p.m.			107.9	25.8	
			July 15		
7:15 a.m.		65.7	100.7		Eyes fully dark.
7:45 p.m.		64.6	85.7	21.8	Yolk diameter 1.9 mm.
			July 16		
7:20 a.m.		64.5	23.1	0.0	Obviously sub-moribund.

It is to be noted that the fish spawning in Lemon Bay were evidently using a site that goes dry at least for intertidal periods and if spawned on a spring tide might be exposed for about two weeks or even more. Also, these eggs have a very long hatching period, something over two weeks under conditions of continual immersion. In view of the fact that they resemble various cyprinodont eggs and there is a variety of morphological reasons to relate the Synentognathi to the Cyprinodontidae, the above conditions suggest that these eggs may be able to stand considerable desiccation in a manner perhaps comparable to those of *Fundulus confluentus* as described by Harrington (1959). It might be that predation on demersal eggs, such as are produced by various species in both these groups, may be less if they are out of or barely in water, rather than submerged to the point of being exposed to the grubbing activity of the large population of *Mugil* and other forms, including a variety of aquatic invertebrates such as the numerous small pagurids, gastropods and small brachyurans. While it is true that the area generally abounds with *Uca*, it is doubtful whether

TABLE 2. REDUCTION OF YOLK WITH DEVELOPMENT IN *Strongylura marina*

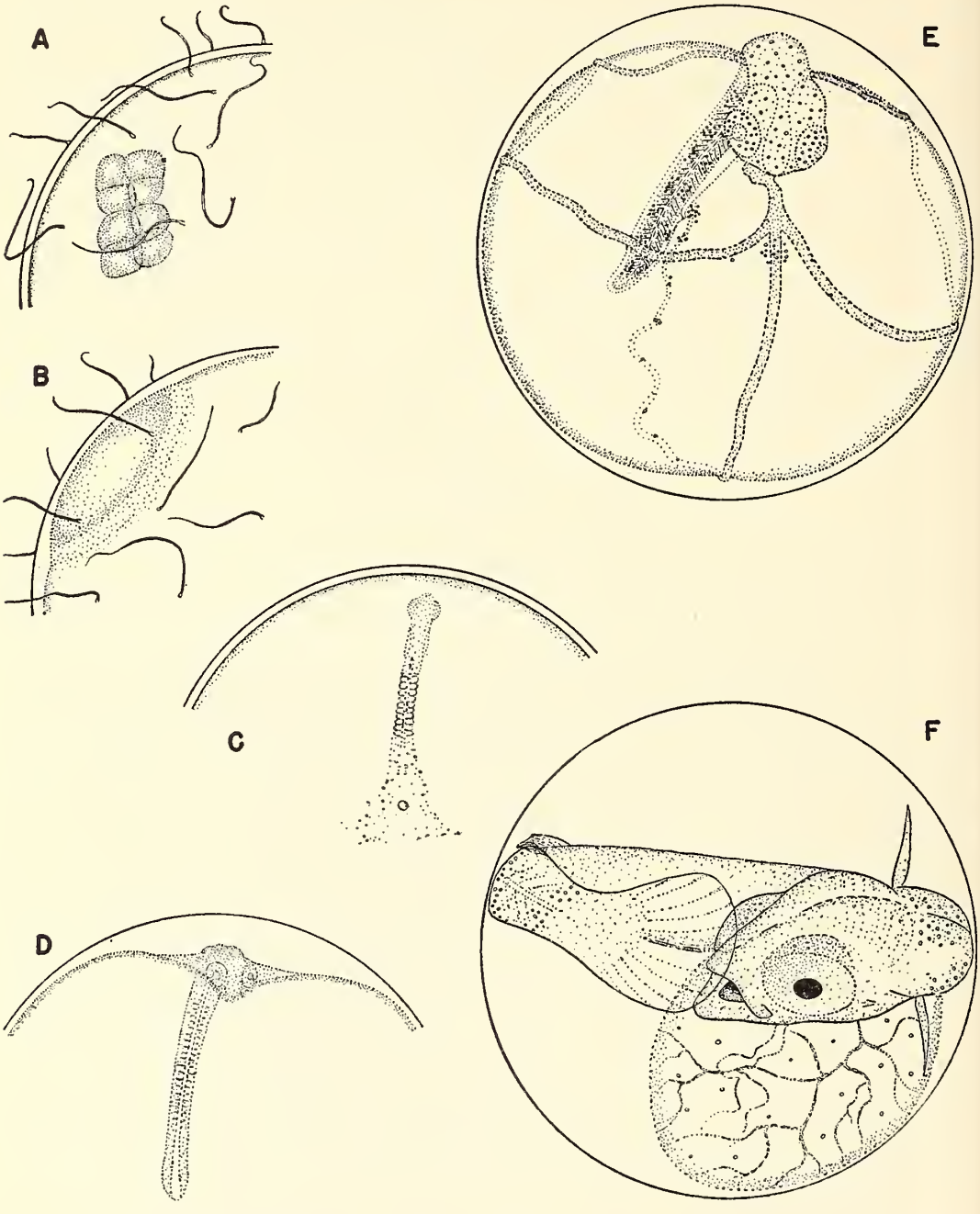
Time is indicated in hours and minutes from fertilization. Volume has been calculated on the assumption that the yolk is spherical, which is as close an approximation as needed for these purposes. Diameter is given in mm. and volume in mm^3.

Time	Yolk Diameter	Yolk Volume
0:00	3.8	28.7
134:40	2.7	10.3
232:05	2.4	7.2
255:05	1.9	3.6

TABLE 3. COMPARISON OF DEVELOPMENT DATA ON *Strongylura notata* WITH THAT OF RYDER'S ON *Strongylura marina*

Time is indicated in hours and minutes from fertilization. Letters in left-hand column refer to illustrations in Text-figure 1. The hours for *S. marina* are to the nearest comparable stages in Ryder (1882).

Stages	*S. notata*	*S. marina*	Difference
"A"	2:20	3:23	1:03
"B"	14:45	10:00	—4:45
"C"	27:10	43:40	16:30
"D"	38:55	70:00	31:05
"E"	71:25	165:30	94:05
"F"	213:15	—	—



TEXT-FIG. 1. Development of eggs of *Strongylura notata*. Times are from fertilization in hours and minutes. The external adhesive threads are shown only in Figs. A and B. The light circles in Figs. E and F represent xanthophores, all in their punctate state. A. Eight-cell stage. 2:20. B. Blastodisc stage. 14:45. C. Segmentation beginning. 27:10. D. Eye formation. 38.55. E. Advanced embryo with the principal blood vessels indicated. 71:25. F. Advanced embryo with much reduced yolk. 213:15.

their numbers or their selectivity for eggs would be as great as that encountered in the totally submerged available sites.

At no time have other species of the smaller inshore species of Belonidae been noted to form aggregations hovering about stands of red mangrove, or any other more or less equivalent shelter, in the manner herein described, although various of them may be swimming by only a short distance away. These include *S. marina, S. ardeola* (Cuvier & Valenciennes) and *S. longleyi* Breder, the spawning sites and habits of which are still unknown.

Summary

1. The eggs of *Strongylura notata* (Poey) go through development typical of their group in a little more than two weeks, at temperatures close to those found in much of their range.

2. Because of the places in which the eggs are spawned, it is suggested that they may be required to withstand considerable desiccation.

3. The spawning behavior is simple and the pairs take a common side-to-side position, forming as much of an "S"-shaped curve as their rather stiff bodies permit.

4. Spawning seems to take place regularly within the tangle formed by the aerial roots of the red mangrove, a situation so sheltered that direct observation is nearly impossible.

5. It appears that spawning always takes place on a lee shore, the least rippling of the surface serving to disperse the spawning groups.

6. No fighting among the males has been noticed, but it would not be surprising if such was found to take place under certain conditions.

Bibliography

Borcea, I.

1933. Nouvelles observations sur les migrations et sur le période de ponte des espèces de poissons migrateurs de la Mer Noire. Ann. Sci. Univ. Jassy, 17: 503-564.

Breder, C. M., Jr.

1932. On the habits and development of certain Atlantic Synentognathi. Papers Tortugas Lab., 28 (1): 1-35. Carnegie Inst. Washington Publ. 435.

Bridges, W.

1942. The story behind a memorandum. Animal Kingdom, 45 (5): 112-114, 119-120.

Dantan, L.

1905. Notes ichthyologiques. Arch. Zool. Exper. Gen., ser. 4, 3: lxi-lxxviii.

Ehrenbaum, E.

1897. Eier und Larven von Fischen der deutschen Bucht. Wiss. Meersuntersuch. Kiel (*Abt. Helgoland*) 1897-1904.

Harrington, R. W., Jr.

1959. Delayed hatching in stranded eggs of marsh killifish, *Fundulus confluentus*. Ecology, 40 (3): 430-437.

Raffaele, F.

1895. Osservazioni sul foglietto epidermico superficiale degli embrioni dei pesci ossei. Mitth. Zool. Stat. Neapel, 12: 169-207.

Ryder, J. A.

1882. Development of the silver gar (*Belone longirostris*), with observations on the genesis of the blood in embryo fishes, and a comparison of fish ova with those of other vertebrates. Bull. U. S. Fish Comm. for 1881, 1: 283-301.

EXPLANATION OF THE PLATES

Plate I

Fig. 1. View from dock showing the shore line and the black mangrove tree which provided an inadequate shelter for the spawning of *Strongylura notata.*

Fig. 2. A spawning aggregation over loose algae in the preliminary stages of jockeying for position.

Plate II

Fig. 3. A troupe leaving the shore side aggregation. In this case there are two females, first and last, and five smaller males between them.

Fig. 4. An actively spawning aggregation with a group of *Mugil* feeding beneath.

Fig. 5. Three eggs with active embryos at 51 hours and 25 minutes from fertilization.

Fig. 6. Edge of one egg by transmitted light, showing the distribution of the adhesive threads.

FIG. 1

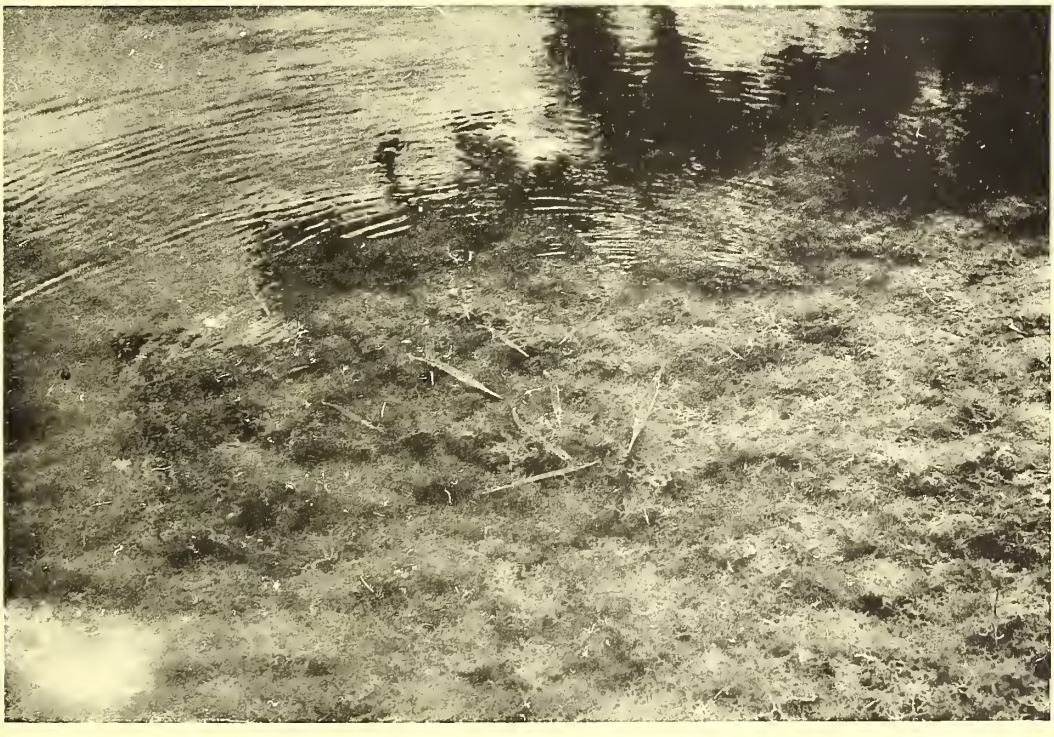


FIG. 2

OBSERVATIONS ON THE SPAWNING BEHAVIOR AND EGG DEVELOPMENT OF STRONGYLURA NOTATA (POEY)

FIG. 3

FIG. 4

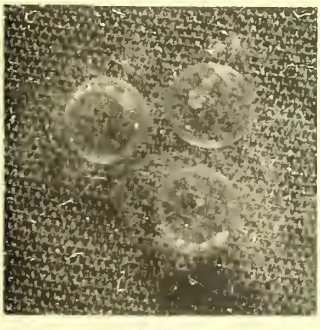


FIG. 5

FIG. 6

OBSERVATIONS ON THE SPAWNING BEHAVIOR AND EGG DEVELOPMENT OF STRONGYLURA NOTATA (POEY)

11

Effects of Four Combinations of Temperature and Daylength on the Ovogenetic Cycle of a Low-latitude Fish, *Fundulus confluentus* Goode & Bean[1]

ROBERT WHITING HARRINGTON, JR.

Entomological Research Center, Florida State Board of Health, Vero Beach

(Text-figures 1-4)

I. INTRODUCTION

EXPERIMENTS on the interplay of ecologic and endocrine factors in fish reproduction indicate that environmental influences on the sex cycle are reflected with greater dependability and particularity in the ovary than in the testis. In fishes as far as known, there is consistent correspondence between phases of the ovogenetic cycle and of the annual reproductive cycle, so that, normally, onset of spawning shortly follows completion of maturation by the vanguard of maturing eggs. Although similar timing holds for the spermatogenetic cycle in some fishes (Harrington, 1957; *et alii*), in others spermiogenesis is completed in autumn with spermiation postponed to spring (Turner, 1919; Kulaev, 1927, 1944; Harrington, 1956), and in still others there is year-round spermatogenesis with no evident change in tempo despite a marked annual ovarian cycle (Ghosh & Kar, 1952). The last condition is approached by *Fundulus confluentus,* and may prove common among low-latitude fishes. Subtler testicular changes, more precisely correlated with the annual emergence of nuptial behavior and color and onset of spawning, supposedly occur, but so far have not been satisfactorily demonstrated. The interstitial Leydig cells, presumed to elaborate androgen, and now deemed widespread among fishes (Marshall & Lofts, 1956), have proved difficult not only to interpret but to recognize in many species. Attention here will be devoted to the ovarian cycle.

Previous experiments on the relation of the environment to the sex cycle of fishes, with one partial exception (*vide infra* Hubbs & Strawn, 1957), were conducted on fishes local to latitudes 41° to 60° North (see complementary reviews of Atz, 1957; Harrington, 1959b). The present experiment was carried out at 27° North Latitude (Vero Beach, Florida), on locally indigenous specimens of the Marsh Killifish, *Fundulus confluentus* Goode & Bean. This species inhabits brackish waters along the whole Florida coastline and northward coastwise to Chesapeake Bay, occasionally entering fresh waters (Miller, 1955), where it is capable of reproducing (Harrington & Haeger, 1958; Harrington, 1959a). It has been reared in freshwater aquaria from egg to maturity and on into a second generation by the writer, but since it frequents salt marshes and mangrove swamps often far removed from fresh waters, oviposition must occur commonly in brackish water, although direct observations are lacking. Its well-known congener, *Fundulus heteroclitus,* ranges southward from Newfoundland, but is subject to the same climatic conditions in the southernmost part of its range. The two species are sympatric from Chesapeake Bay to the Matanzas River, in northeastern Florida, or throughout the range of *F. confluentus,* if the closely similar *F. grandis* be judged a subspecies of *F. heteroclitus,* which remains to be decided (Brown, 1954, 1957). Experiments concerning effects of extrinsic factors on reproduction in *Fundulus* have been confined to northern representatives of *F. heteroclitus* (Matthews, 1939; Burger, 1939, 1940), and have dealt exclusively with the spermatogenetic cycle. Since these were without specific reference to nuptial behavior and color or to sper-

[1] Contribution No. 74, Entomological Research Center, Florida State Board of Health, aided by grant No. 5415, National Institutes of Health, U. S. Public Health Service.

miation, and since the relation of the environment to the ovarian cycle was not studied, intrageneric comparisons with reference to latitude will not be possible.

The most appropriate season for testing effects of environmental factors on successive phases of the annual reproductive cycle is the non-breeding season, at the outset of which the ovaries—and in many fishes the testes also—undergo their maximum annual regression. This season is strikingly shorter at low than at high latitudes for fishes as for many other vertebrates. In Florida, *F. confluentus* evidently spawns soon after the end of January and continues into October, a breeding season much the same as that of the Pigmy Seahorse, *Hippocampus zosterae,* so instructively worked out in Florida by Strawn (1958). In contrast, the breeding season of *F. heteroclitus* in New England is June to early August (Bigelow & Schroeder, 1953). This leaves annually a non-breeding season of almost nine months for *F. heteroclitus* in New England but one of scarcely three months for *F. confluentus* in Florida, and brings to the fore the problem of the so-called refractory or postspawning period of fishes (*cf.* Harrington, 1957, 1959b).

The brief annual hiatus between successive breeding seasons at low latitudes affords little time for experimental maneuver. The duration of environmental conditions typical of the non-breeding season will be further curtailed by imposing within it variant experimental conditions, some of which might be expected to induce unseasonable changes in the course of maturation within the range of oocyte phases. Quantitative measurement of egg maturation, found useful in experiments at high latitudes (Bullough, 1939; Harrington, 1956, 1957), is all the more needed for effective analysis of results from experiments at low latitudes, because of possibly greater asynchrony in ovarian maturation among fish exposed to experimental conditions within the shorter annual interval between normal breeding seasons.

Before describing results of the present experiment, it will be necessary to define a sequence of oocyte phases as reference points for measuring quantitatively the stage-by-stage progress of ovogenesis within each group of fish exposed to a particular temperature-daylength combination.

II. Material and Methods

The experiment was begun December 15 and ended 45 days later, on January 31. Unforeseen difficulties prevented starting it December 1, as planned, but whether this would have sharpened the contrast in maturity at the end of the experiment between the most advanced experimental fish and those in the wild can only be conjectured. It appears most likely that had the experimental period been further postponed after the preceding spawning season, it would have ended so late as to greatly reduce the potential maximum contrast between a possible precocious maturation under any of the variant experimental conditions and the status of maturation in the wild.

Marsh Killifish were allocated to the four 20-gallon experimental aquaria on December 15, after having been acclimated for three days by gradually replacing the brackish water in which they had been collected with fresh. Since this species was found to spawn in fresh water both in aquaria and in the wild, the fish were changed to fresh water to avoid the further elaboration of apparatus required to minimize the tendency of brackish water in aquaria to become foul. Others of these acclimated fish were sacrificed the same day (December 15 controls). On January 31, fish fresh from the wild (January 31 controls) were sacrificed together with all fish in the experimental aquaria.

Two aquaria were kept in one bioclimatic room and two in another. Both rooms were sealed from daylight and regulated to maintain a water temperature of 15 ± 1° C. In each room, one aquarium was left at 15 ± 1° C. and the other kept at 30 ± 1° C. by means of a thermostatically-controlled aquarium heater. All illumination was from two fluorescent lamps (Westinghouse 40-watt Daylight), suspended 30 inches above each aquarium bottom; the light intensity at the bottom of the aquarium when empty was 753 lux. One room was illuminated for seven and the other for 15 hours each day. Each aquarium was supplied with an aerator and a charcoal filter; the fresh water filling it was not changed during the experiment, but well water was added to compensate for evaporation. Plants tolerating dim light (*Cryptocoryne* spp.) were rooted in the bottom sand.

This cover failed to allay the initial excitement of the wild fish, and a few bruised their snouts against the glass. When the killifish became habituated to captivity, all injured ones were removed. There was no mortality or sickness after the removal of the fish injured at the outset of the experiment. The numbers of female killifish in each of the four aquaria at the end of the experiment were 15, 17, 15 and 14, respectively (Table 2). In addition, each aquarium contained eight to ten males.

The fish were fed *ad libitum* once daily on

live mosquito larvae (*Aëdes* spp.), alternated with live daphnia and supplemented with mosquito larvae frozen in small blocks. The latter were ingested gradually as they fell from the melting blocks. Live food in excess of immediate demands remained in the aquaria to be consumed later, affording ample food for all fish by mitigating the influence of social dominance on feeding.

The standard lengths of all fish connected with the experiment were measured with vernier calipers to the nearest tenth of a millimeter. Originally selected for adult size, all proved much larger than the smallest that have spawned viable young in our aquaria. Each fish was weighed on a beam balance to the nearest hundredth of a gram, after removal of surface water with filter paper. Upon dissection and after remaining briefly on filter paper, the gonads were rapidly weighed on a Roller-Smith balance to the nearest two-tenths of a milligram, then fixed in Bouin's solution. After dehydration in an alcohol series, clearing in xylol, and transfer to cedar oil, the ovaries while in oil were teased apart, and the largest 50 egg diameters measured with an ocular micrometer. The testes were sectioned and stained with Heidenhain's iron haematoxylin, but as anticipated above, showed no obvious weight, size or histological differences, seasonal or otherwise, and will be left out of further account. If there is a seasonal change in the testes of *F. confluentus,* it is even more transitory than in those of *Oryzias* (Egami, 1956) and of little diagnostic utility in studies like the present one. In this respect *F. confluentus* differs from *F. heteroclitus,* the northern representatives of which, at least, have an obvious seasonal testicular cycle (Matthews, 1938; *et alii*).

Nuclear diameters as well as egg diameters were measured on a series of eggs from a representative fish of each experimental group and of both December 15 and January 31 controls. The nucleus is easily measured in cleared eggs of all phases of maturation up to the stage of yolk consolidation, for which there are no data on nuclear diameters. Since the distribution of nuclear diameters at each egg diameter was similar for corresponding egg diameters of different ovaries, the data for the fish of all groups were pooled (Table 1 and Text-fig. 1). Both egg and nuclear diameters were measured along whatever axis fell at random along the micrometer scale, a procedure sanctioned by the statistical analysis of Clark (1925). Sections of additional ovaries, fixed and stained the same as the testes, were examined to determine the approximate egg-diameter ranges corresponding with successive morphological phases of the ovogenetic progression.

The nucleoplasmic index at each egg diameter was computed from the mean nuclear diameter at each egg diameter, by the conventional formula, in which nuclear volume is divided by the difference between egg volume and nuclear volume. Egg and nucleus were treated as spheres, their volumes being computed from their diameters by the formula, $\pi D^3/6$, or $0.524\ D^3$. The gonosomatic index of each fish was obtained by multiplying the weight of the pair of gonads by 100, and dividing by the body weight.

III. Oocyte Phases

A. Defined by Egg Diameters Related to Changing Nuclear Diameters and to Yolk Consolidation

The changes in nuclear diameter with increasing egg size are recorded in Text-fig. 1. Each unit on the egg-diameter scale, along the x-axis, and of the nuclear-diameter scale, along the y-axis, equals 23 microns. In Text-fig. 1a, the distribution of empirical nuclear diameters at each egg diameter is plotted as a vertical row of dots. Each dot, according to its size, represents from one to 11 nuclei of the same diameter. In Text-fig. 1b, each hollow dot, determining the heavy line, records the mean nuclear diameter at a particular egg diameter. Each solid dot, determining the light line, records the nucleoplasmic index computed from a particular egg diameter and its corresponding mean nuclear diameter. The scale of nucleoplasmic indices is to the right. The data plotted in Text-fig. 1b are correlated with additional data in Table 1.

With graded increase in egg size, the nuclear diameter first undergoes a rapid increase, followed by a gradual decrease, and then tends to level off. Eggs of diameters greater than shown in Text-fig. 1 are in an advanced phase of maturation and so obscured by yolk that in cleared eggs the nucleus cannot be measured. With a shift in the growth differential between egg and nuclear diameters, the nucleoplasmic index begins its sharp decline somewhat before the rapidly enlarging nucleus reaches maximum diameter; the decline continues while the nuclear diameter is subsequently on the decrease, and approaches its minimum asymptotically as the nuclear diameter levels off. Although increase in egg diameter is a function of time, the egg-diameter scale conforms only to time sequence, not to time intervals.

At the outset of the series of successively larger eggs through which the nucleus pro-

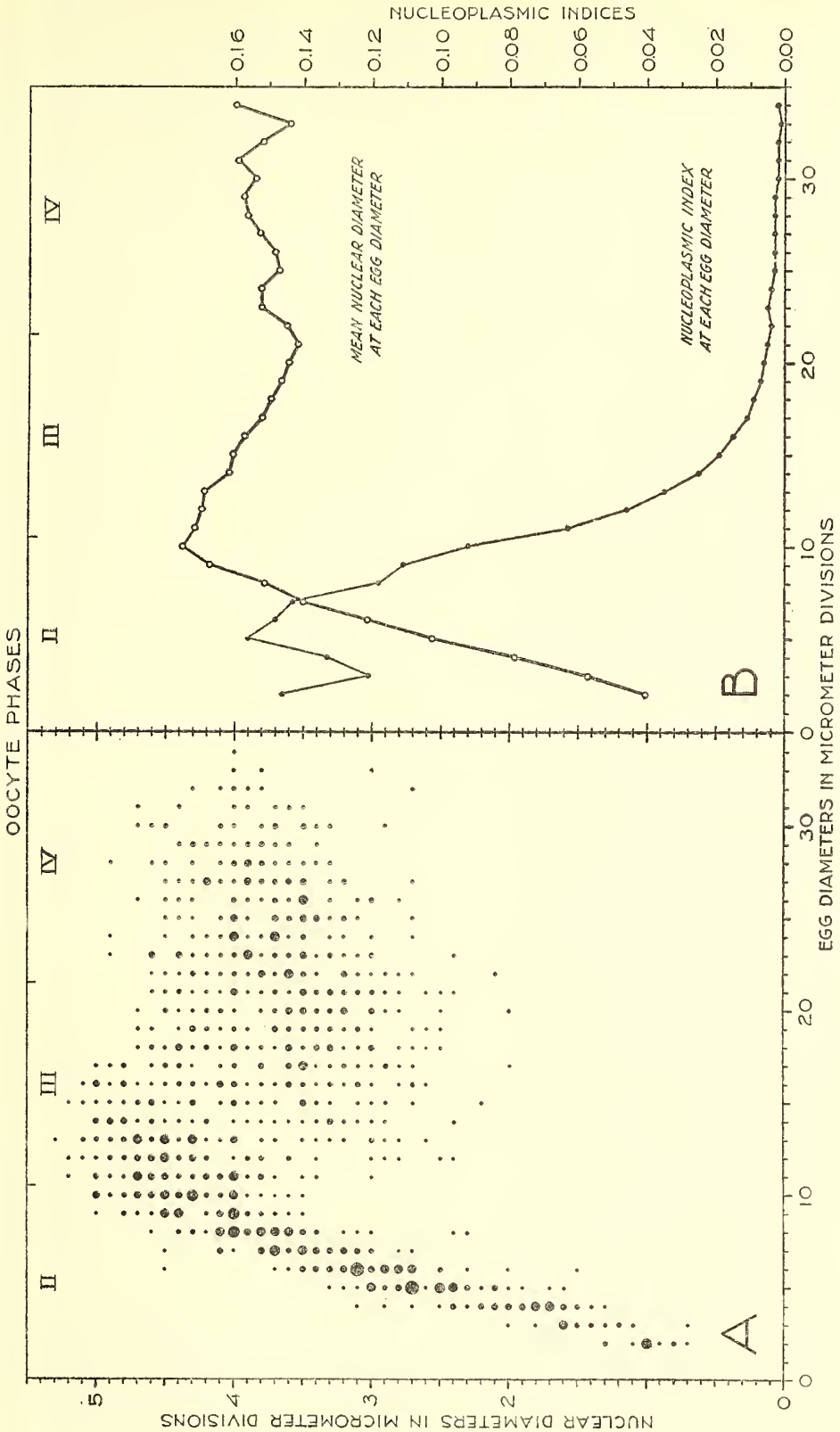
Table 1. The Correlation of Nuclear Diameter and Nucleoplasmic Index with Egg Diameter in Maturing Eggs of *Fundulus confluentus*. Compare Text-fig. 1.

Egg[1] Diameter	Number of Eggs Measured	Corresponding Nuclear Diameters[1]				Nucleoplasmic Index (Computed from Mean Nuclear Diameter)	Oocyte Phase
		Mean	Median	Max.	Min.		
2	18	1.01	1.00	1.3	0.7	0.146	
3	23	1.43	1.47	2.0	0.7	0.121	
4	52	1.96	1.91	3.1	1.3	0.133	
5	60	2.56	2.55	3.3	1.6	0.156	II
6	61	3.03	3.06	4.5	1.5	0.148	
7	52	3.50	3.49	4.5	2.7	0.143	
8	60	3.78	3.82	4.6	2.3	0.118	
9	48	4.18	4.11	5.0	3.5	0.111	
10	57	4.38	4.38	5.0	3.5	0.092	
11	55	4.29	4.30	5.2	3.0	0.063	
12	53	4.24	4.43*	5.2	2.4	0.046	
13	56	4.22	4.35*	5.3	2.6	0.035	
14	41	4.04	4.20*	5.0	2.4	0.025	
15	43	4.01	4.04	5.2	2.2	0.019	
16	58	3.93	4.00	5.1	2.6	0.015	III
17	49	3.80	3.64*	5.0	2.0	0.011	
18	56	3.74	3.67*	4.7	2.5	0.009	
19	44	3.66	3.68	4.7	2.5	0.007	
20	50	3.61	3.67	4.7	2.0	0.006	
21	50	3.54	3.49	4.6	2.4	0.005	
22	42	3.62	3.65	4.6	2.1	0.004	
23	47	3.81	3.86	4.9	2.4	0.005	
24	31	3.81	3.78	4.9	2.7	0.004	
25	35	3.68	3.63	4.5	2.7	0.003	
26	27	3.71	3.67	4.7	2.8	0.003	
27	38	3.82	3.85	4.5	2.7	0.003	
28	22	3.91	3.89	4.9	3.3	0.003	IV
29	19	3.94	3.90	4.4	3.4	0.003	
30	17	3.85	3.74	4.7	2.9	0.002	
31	9	3.98	3.95	4.7	3.5	0.002	
32	6	3.80	3.50	4.3	2.7	0.002	
33	3	3.60		4.0	3.0	0.001	
34	1	4.00		4.0	4.0	0.002	

[1] One unit = 23 μ. * See text.

gressively decreases in size (Text-fig. 1b), the empirical data show a pronounced increase in the range of nuclear diameters (Text-fig. 1a). Nuclear diameter distributions are at first skewed toward the smaller diameters, then a less evident countertendency sets in (*cf.* also the starred medians in Table 1), but there is no consistent skewness after the mean nuclear diameter reaches its minimum. The spread of nuclear diameter distributions is no doubt somewhat enhanced by the measurement at random along axes of ovoid nuclei and by the incidence of aspherical eggs, although the maturing ovaries of the Marsh Killifish are less compact and developing eggs depart less from spherical shape than in other fishes examined by the writer. The rather abruptly increased spread in nuclear diameter distributions is ascribable mainly to relatively short-time changes in nuclear volume. The present data do not rule out, but are insufficient to demonstrate, pulsations in the volumes of individual nuclei at a particular egg diameter, accompanying the gradual diminution of the mean nuclear diameter.

Hereafter, eggs with diameters ranging up to 10.9 units will be referred to oocyte phase II within which the nucleus increases in size; eggs with diameters between 11 and 21.0 units, to oocyte phase III within which the nucleus decreases in size; eggs with diameters from 22 to about 34 units, to oocyte phase IV within which nuclear diameters tend to level off; and all larger eggs, to oocyte phase V which includes mature eggs (compare Tables 2-3 and Text-figs. 2-4).



TEXT-FIG. 1. Interrelations of oocyte phase, egg diameter and nuclear diameter in *Fundulus confluentus*. One micrometer division = 23 μ. Compare Table 1. (A) Range of nuclear diameters at each egg diameter. Each dot, according to size, records from one to 11 measurements. (B) Mean nuclear diameter and nucleoplasmic index at each egg diameter.

B. Significance

The oocyte phases defined above approximate those given the same numerical designations by Detlaf & Ginzburg (1954), but established on a morphological basis (*cf.* Ivanov & Dodzina, 1957) by Meien (1939), who named them, respectively, the one-layered follicle phase, the phase of the primary accumulation of yolk, the phase of the filling up of the oocyte with yolk, and the phase of the ripe oocyte. In this classification, oocyte phase I is known as the juvenile phase and is characterized by, among other things, an at best incomplete follicular epithelium in which the nuclei of the epithelial cells investing the oocyte are remote from each other. Based on complexes of these oocyte phases, a series of ovarian maturation stages are defined by Russian workers, which will be referred to hereafter merely as ovarian stages. To simplify, the most advanced oocytes in *ovarian stages* I to V are in *oocyte phases* I to V, respectively. With allowance for differences among fish taxa and difficulties of precisely establishing the extreme diameter limits of each oocyte phase, our oocyte phases correspond with those similarly designated by Detlaf & Ginzburg (1954) as oocyte phases II to V. Trusov (1947) subdivides ovarian stage IV in the European Pike-perch *(Lucioperca)* into IV-A, IV-B, and IV-C (actually labelled by the first three letters of the Cyrillic alphabet), in effect limiting stage V to that condition of the ovary in which the ripest eggs have left their follicles. The transition from late oocyte phase IV to phase V in *Fundulus confluentus* is so slight and rapid that the exact boundary between late ovarian stage IV and stage V is of little import in the present experiment, and is mentioned only to underscore the fact that our oocyte phase V includes oocytes corresponding to those typifying both Trusov's ovarian stages IV-C and V. Ovarian stage VI is characterized by the presence of empty (atretic) follicles, and is thus first encountered after onset of spawning. Our oocyte phases are numbered to permit comparisons with the apposite ovarian stages cited in the extensive Russian literature on fish reproduction. The writer otherwise would have followed the classification of fish oocyte phases used by Yamamoto (1956a), which is based on more thorough cytomorphological and cytochemical studies. Moreover, the present investigation concerns responses throughout a succession of oocyte phases, while the Russian nomenclature emphasizes ovarian stages. Although each ovarian stage is based on a *complex* of oocyte phases, this nomenclature diverts attention from the earlier oocyte phases to the most advanced oocyte phase of that complex of oocyte phases characterizing a particular ovarian stage.

Oocyte phases I and II belong to the lesser growth period of the oocyte, all later phases to the greater growth period, or period of more rapid growth (Meien, 1939; and subsequent Russian authors). These two growth periods evidently correspond with the primary and secondary growth periods (*e.g.* of Bullough, 1939) or the growth periods 1 and 2 (of Marza *et al.*, 1937). The secondary growth period is described and illustrated for *Fundulus heteroclitus* by Marza *et al.* (1937) as period 2, of which phases A_1 and A_2 approximate our oocyte phase III, phases B_1 and B_2, our oocyte phase IV, and phase B_3, our oocyte phase V. According to Sakun (1957), the period of trophoplasmatic growth includes the phase of primary accumulation of yolk, in which vacuoles are formed in the cytoplasm (our oocyte phase III), and the phase of the accumulation of yolk in the form of lipid-containing granules (our oocyte phase IV). Konopacka (1935) and Yamamoto (1955a, b; 1956e, f, g; 1958) specify that vitellogenesis proper begins with the first appearance of vacuoles. Mas (1952) equates these with his "plagues claires" and with the "proteinaceous yolk vesicles" described in *F. heteroclitus* eggs at the outset of growth period 2 (Guthrie, 1928, 1929; Marza *et al.*, 1937). Kazanskii (1951) describes ovaries in stage III, in which the advanced eggs had fluid-filled vacuoles but as yet no lumps of yolk. Bullough (1939) notes that growth beyond the primary growth period is coincident both with loss of cytoplasmic basophilia and with the appearance of vacuoles, in connection with which "yolk droplets" finally arise. Chopra (1958) describes clear vacuoles giving rise to 'vacuolar yolk,' which he equates with the "yolk vesicles," the name used for these vacuoles by Yamamoto (1955a *et seq.*), who found them to be chiefly composed of polysaccharides and who believed them finally to give rise to the cortical alveoli, which at fertilization are extruded into the interspace between plasma membrane and egg membrane.

The consensus of these works is that the beginning of the greater (secondary) growth period of the oocyte coincides with the onset of vitellogenesis, for which the *ab initio* appearance of cytoplasmic vacuoles (yolk vesicles) is diagnostic.

In sectioned eggs of *F. confluentus,* these yolk vesicles are first seen at egg diameters of about 10 units (*cf.* Text-fig. 1b), the egg diameter above which the nucleus begins to diminish in size, suggesting that reduction in

nuclear diameter is related to onset of vitellogenesis as expressed in the changes occurring within oocyte phase III, the phase of primary accumulation of yolk (yolk vesicle phase). The antecedent onset of the decline in the nucleoplasmic index (Text-fig. 1b) reflects a change in the differential between egg and nuclear growth rates, which may prove equally significant in the chain of events correlated with onset of vitellogenesis. It is soon after this that the yolk vesicles appear and the cytoplasm loses its basophilia (*cf.* preceding paragraphs).

The phenomenon of nuclear diminution recorded in Text-fig. 1 seems otherwise to have escaped notice in fish eggs. It would be difficult to detect from measurements on sectioned ovaries. Subramaniam & Aiyar (1935) noted a "progressive reduction in the size of the *nucleoli* [italics ours] with increase in the size of the fat-globules" in *Acentrogobius neilli.* Singh & Boyle (1938) twice misquoted this as a reduction in the size of the *nuclei,* which they associated with expulsion into the cytoplasm of nucleolar substances, inferred from their own studies to initiate the formation of the vacuoles (yolk vesicles). The validity of such extrusions in fish oocytes has been denied (Nath *et al.,* 1944; Nath, 1957), but Yamamoto (1955a, b; 1956b; 1958) presents topographical and cytochemical evidence of their occurrence and participation in yolk vesicle formation. According to Bonhag (1958), on the other hand, the general theory that nucleolar emissions play a role in yolk production awaits adjudication by ultramodern techniques. More recently, however, Hsu & Lou (1959) have demonstrated by time-lapse cinematography the extrusion of nucleolar material in mouse melanoma cells, in time sequences showing discontinuous output (Nuclear Pump Action). Moreover, they found that fresh nutrient was essential to the process, which points to a requirement for ribonucleic acid material from the nucleus for the synthesis of new material in the cytoplasm (*cf.* comments of discussants, *loc. cit.*). Of further interest in this connection is the hypothesis of Pantelouris (1958), derived from the study of radioisotope-labelled newt oocytes, that a protein migration from cytoplasm to nucleus predominates before vitellogenesis and migration in the opposite direction occurs during vitellogenesis.

IV. Experimental Results

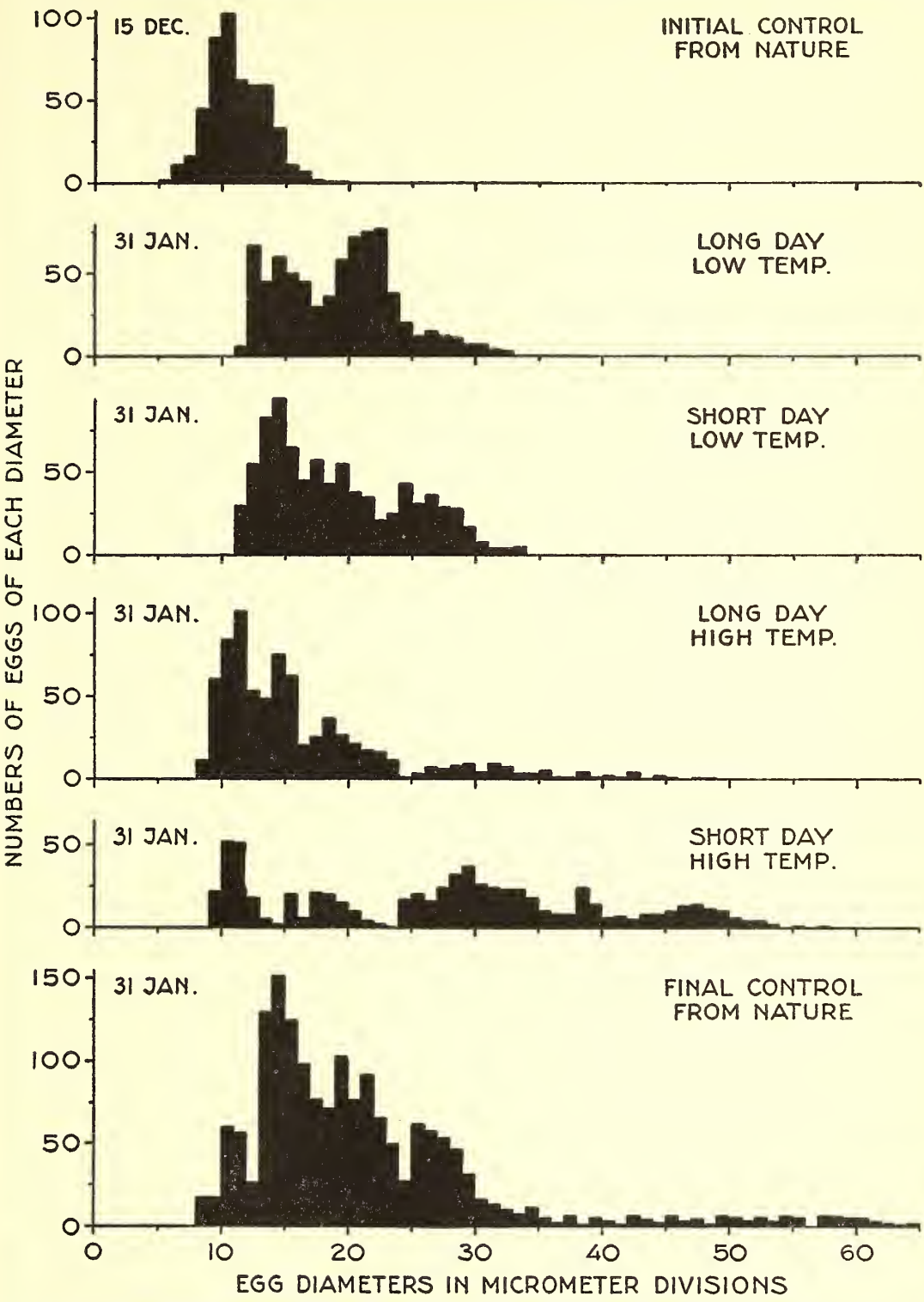
At the beginning of the experiment, the ovaries were in stages II and III, their largest oocytes being about equally divided between oocyte phases II and III (Table 2 and Text-fig. 4). The experiment was ended when the largest oocytes in the ovaries of the ripest fish were in our phase V (*vide supra* Oocyte Phases). Observation was intensified toward the end of the experiment to assure its termination before spawning began. Examination of the ovaries later confirmed that no fish had spawned. The experimental results can therefore be evaluated in terms of the progress of the largest 50 eggs per fish along the scale of egg diameters corresponding to oocyte phases II through V, paralleling the like-numbered ovarian stages, as well as with reference to gonosomatic index.

The frequency distributions of the largest 50 eggs per fish are pooled for each experimental group in Table 2 and illustrated in Text-fig. 2 for comparison with analogous histograms in the literature (*cf.* Bullough, 1939; Harrington, 1956, 1957, 1959b). The range of the largest 50 egg diameters is separately recorded for each fish as a vertical line in Text-fig. 3. The ranges within each experimental group are disposed in an ascending series with the more mature fish represented to the right and the more mature eggs toward the top. The scale of egg diameters is to the left. The hollow dot related to each vertical line records the gonosomatic index of the same fish, with the scale of gonosomatic indices to the right, in which the higher the value, the more mature the fish. Table 3 gives the frequency distributions of the largest 50 egg diameters separately for each fish of the two high-temperature groups, of which one was subject to a long and the other to a short daily photoperiod. Text-fig. 4 shows the percentages of all eggs measured for each experimental group (the group sum of the largest 50 per fish) found in oocyte phases II to V, respectively, as computed from the data in Table 2. To shorten circumlocution, the largest 50 egg diameters per fish will often be referred to as the vanguard eggs.

The general character of the group response to each of the four variant treatments, as well as the status of maturation in the wild at the beginning and end of the experiment, is apparent from Table 2 and Text-fig. 2. In contrast to experimental constant temperatures of 15° C. or 30° C. and fixed daylengths of 7 hours or 15 hours, the terminal (January 31) controls were subject in the wild to fluctuating temperatures closer to the low than to the high experimental temperature and to daylengths closer to the short than to the long experimental daylength. In December, the average air temperature locally was 19.1° C. (av. daily max., 23.3° C.; av. daily min., 13.1° C.); in January, it was 18.9° C. (av. daily max., 24.4° C.; av. daily min., 11.7° C.). During the 45-day experi-

TABLE 2. EFFECTS OF DAYLENGTH AND TEMPERATURE ON THE MATURITY OF *Fundulus confluentus*. The frequency distributions of the largest 50 egg diameters per fish pooled for each group. One egg-diameter unit = 23μ. Compare Text-fig. 2.

Egg Diameters	Control Dec. 15 (10 Fish)	15° C.		30° C.		Control Jan. 31 (33 Fish)	Oocyte Phases
		15-hr Day. (15 Fish)	7-hr. Day (17 Fish)	15-hr Day. (15 Fish)	7-hr. Day (14 Fish)		
5	2						
6	11						
7	16						
8	n= 45	n=	n=	n= 11	n=	n= 17	II
9	265 88	0	0	155 60	74 22	93 17	
10	103			84	52	59	
11	62	6	28	101	51	56	
12	59	67	55	53	18	26	
13	59	45	83	48	5	129	
14	33	60	95	75	2	151	
15	n= 11	n= 50	n= 65	n= 62	n= 20	n= 124	III
16	235 7	544 45	599 45	484 20	172 6	999 97	
17	2	30	57	25	21	76	
18	1	36	43	36	20	71	
19	1	58	55	26	15	102	
20		72	38	21	10	76	
21		75	35	17	4	91	
22		77	21	16	2	65	
23		38	25	11		49	
24		20	43	1	17	27	
25		12	31	3	20	61	
26		15	36	7	16	57	IV
27	n=	n= 12	n= 29	n= 6	n= 24	n= 53	
28	0	206 11	251 28	87 8	262 32	446 46	
29		7	17	9	37	31	
30		7	8	4	26	16	
31		4	4	9	24	13	
32		3	4	7	23	10	
33			5	3	23	7	
34				3	18	11	
35				5	10	5	
36				1	8	2	
37	n=	n=	n=	n= 1	n= 8	n= 6	V
38	0	0	0	24 4	192 24	112 1	
39				1	14	5	
40				2	6	3	
41				1	7	1	
42				4	5	6	
43					8	4	
44				2	8	2	
45				1	10	6	
46					13	3	
47				1	14	4	
48				1	11	1	
49					10	6	
50					6	5	
51					4	3	
52					4	5	
53					2	3	
54						6	
55					1	5	
56							
57					1	6	
58-64						24	



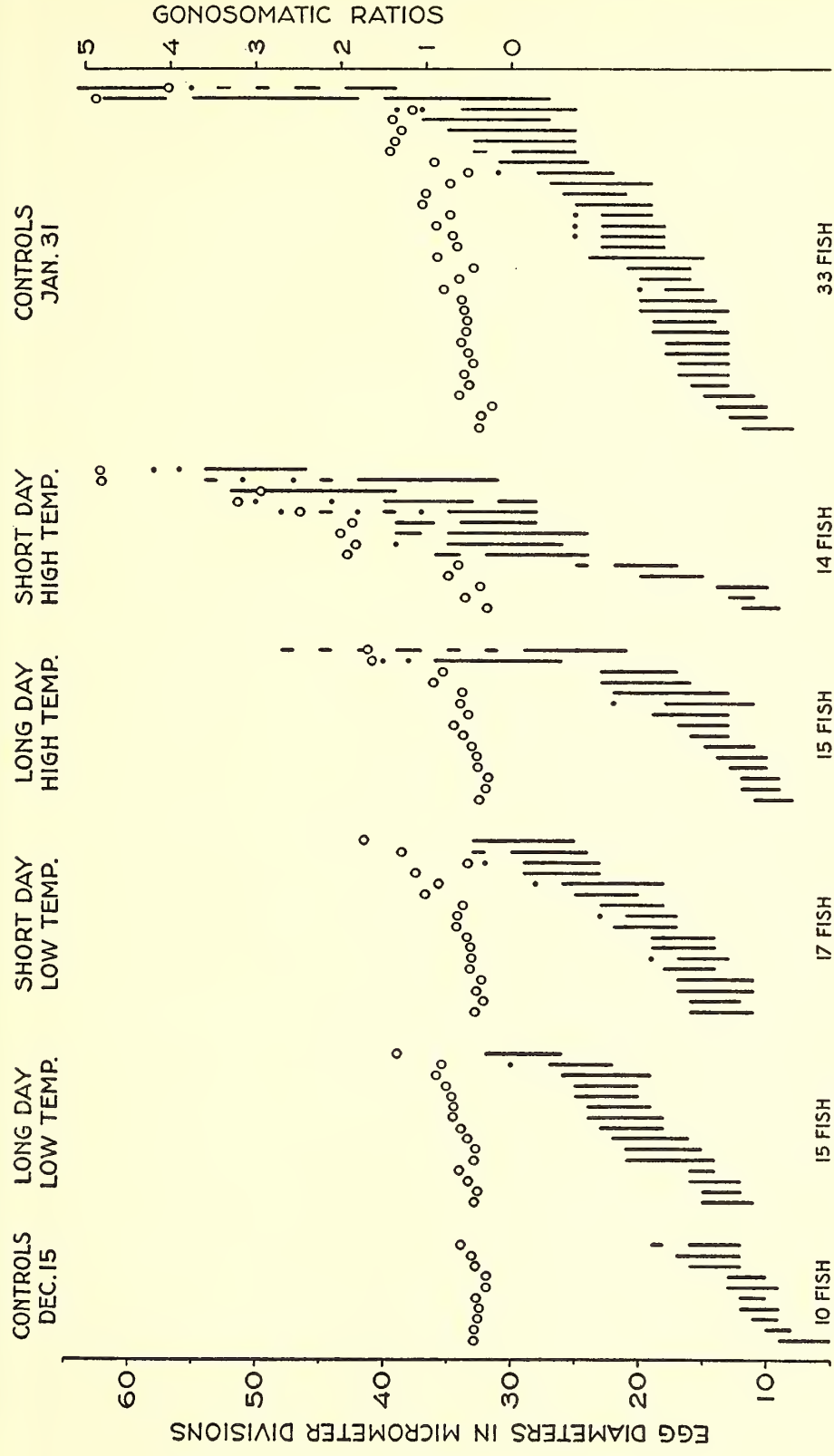
TEXT-FIG. 2. Histograms of effects of daylength and temperature on the ovaries of *Fundulus confluentus*. The diameters of the largest 50 eggs per fish pooled for each group are plotted from data in Table 2. One micrometer division = 23 μ.

TABLE 3. EFFECTS OF DAYLENGTH ON THE MATURITY OF *Fundulus confluentus* AT 30° C.

Egg[1] Diameters	Frequencies of the Largest 50 Egg-Diameters per Fish																												
	15-hour Day (15 Fish)															7-hour Day (14 Fish)													
8	11																												
	32	19	9													22													
10	6	19	22	27	10											25	27												
	1	9	15	13	26	23	14									2	16	33											
12		3	4	9	10	15	12									1	3	14											
				1	2	8	7	10	9	7	4						2	3											
14					2	2	7	17	18	17	12						2												
						2	4	16	17	10	13								20										
16							1	5	6	5	2	1							6										
							1	2		7	4	10	1						8	13									
18							3			3	4	13	13						10	10									
										1	4	8	13						5	10									
20											3	6	12						1	9									
											1	7	5	4						4									
22							1				3	3	5	4						2									
												2	1	8															
24														1						1	12	4							
														3						1	11	8							
26														2	5						8	1	7						
														1	5						6	8	10						
28														1	7						5	3	5	9	7	2			
														5	4						1	4	10	7	8	7			
30															4						2	3	4	9	3	5			
														2	7						1	2	4	7	5	4	1		
32														3	4						1	3	4	5	5		5		
															3							6	2	4	5	2	4		
34														1	2						1	2	1	4	1	5	4		
														2	3						1	3	1		3	4	6		
36															1						1			1		3	5		
														1								1		2	2	2	1		
38														1	3							1		1		4	1		
														1								1	1	1	6	5	4	6	
40															2										1	4	5	4	
														1													4	2	
42														4											1		2	4	
																												5	
44														2											1	2	2	3	
														1											1		1	6	
46																												6	4
														1													1	3	9
48														1											1			3	10
																												1	10
50																										1		5	4
																											1	1	4
52																												1	3
																											1		3
54																											1		1
56																													1
58																													1

[1] One unit = 23 μ.

mental period, natural daylengths declined from 10.5 to 10.4, then increased to 10.9 hours. The January control group resembles the two groups at high temperature in that the vanguard eggs comprise oocytes of phases II and V as well as intermediate ones. In only two of the 33 control fish have eggs reached phase V, however, and in only three are the van-



TEXT-FIG. 3. Effects of daylength and temperature on the ovaries of individual *Fundulus confluentus*. Each vertical line spans the largest 50 egg diameters of one fish; the related hollow dot records the gonosomatic index of the same fish. One micrometer division = 23 μ.

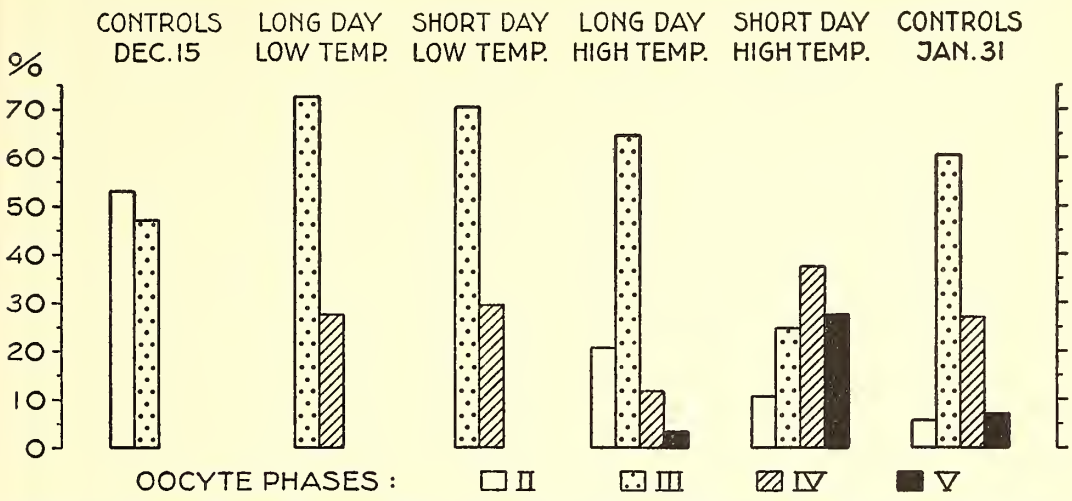
guard eggs represented in phase II (Text-fig. 3). The distribution of the vanguard eggs in the January 31 control group is otherwise much like that of both low-temperature groups, and may be regarded tentatively as intermediate between that of the short-day, low-temperature group and that of the long-day, high-temperature group.

The two low-temperature groups are closely similar, and differ in two respects from all other groups at the end of the experiment. The vanguard eggs in both have without exception passed entirely out of phase II into phase III or beyond, but none have reached phase V. In both high-temperature groups, on the contrary, vanguard eggs are represented by both phase II and phase V oocytes, as in the January 31 control group. The distribution of these maturing eggs, moreover, is suggestive of opposing tendencies, *viz.* a lag in development at smaller diameters and a speed-up at greater ones. Of the two groups at high temperature, the one subject to short days is by far the more advanced in maturity and, as will be demonstrated from several aspects, is by far the most advanced group in the experiment. At low temperature, on the other hand, there seems to be little if any difference in maturation with contrasting daylengths. The slight advance (within phase IV, compare Text-figs. 2 and 3) of short-day over long-day fish at low temperature derives its sole significance, if any, by analogy with the marked advance under short days at high temperature, since it is not statistically significant.

The above disposition of the experimental data reflects the extent and amplitude of each group reaction, and permits group-to-group comparison, only moderately blurred by the variation in numbers of fish per group (Table 2). It does, however, conceal the responses of individual fish. This omission is corrected by Text-fig. 3, in which the extent of the response of each fish is measured by the position and length of the vertical line spanning the range of its largest 50 egg diameters. The amplitude of its response is roughly expressed by its gonosomatic index, but despite general conformity between gonosomatic indices and later phases of egg maturation, the gonosomatic index proves to be a tardy indicator of the course of ovogenesis (Text-fig. 3), effectively registering progress in maturation only beyond oocyte phase III (*cf.* Text-fig. 1). Quantitative analysis of egg diameters is required to assess the amplitude and extent of maturation through oocyte phases I-III in this species, although in its much larger congener, *F. heteroclitus,* this may not prove necessary.

Text-fig. 3 also discloses the staggered pattern of responses by the fish within each group exposed to the same daylength-temperature combination. It further shows that in the low-temperature fish, the ovaries were either in stage III or IV, and that these fish can be arranged in an evenly-graded series with respect to maximum egg diameters attained in each, within the range of diameters encompassed by oocyte phases III to mid-IV. In contrast, the ovaries of both groups at high temperature (*cf.* Table 3) were either in stage III or V. Some of the stage-III ovaries contained vanguard eggs in oocyte phase II as well as in oocyte phase III, and their largest eggs were at most on the border line between phases III and IV, unlike the stage-III ovaries of the low-temperature fish. Most of the stage-V ovaries had vanguard eggs in oocyte phase IV in addition to those in oocyte phase V. The only high-temperature fish that can be evenly graded with reference to maximum egg diameters attained are those with no eggs in phase V. Beginning with phase IV, there is for the first time a sudden, pronounced extension of the distributional spread of the vanguard egg diameters (Tables 2-3 and Text-figs. 2-3). This sudden increase in the range of diameters comprehended by the largest 50 eggs no doubt reflects an abrupt change in the growth rate of larger eggs as they come under influences rapidly leading to definitive egg size (*cf.* figures of Harrington, 1956, 1957, 1959b; and Text-fig. 1 of Yamamoto, 1956a). In some fish (Text-fig. 3 and Table 3), these eggs range from diameters appropriate to the outset of phase IV to those equal or almost equal to mature eggs. This draws attention to the unprotracted ranges of the vanguard egg diameters in the low-temperature fish, including even those in which vanguard eggs have entered oocyte phase IV (Text-fig. 3).

When eggs that were measured in 70% alcohol after fixation in Bouin's solution are remeasured in cedar oil after clearing, the largest ones are found to have shrunk as much as 7-8 units on the present scale, or about 0.16-0.19 mm. Shrinkage is progressively less at smaller diameters, becoming negligible toward the smallest diameters of oocyte phase IV. This places the largest eggs in the experiment at the definitive size for the species. Living fertile eggs of *F. confluentus* measure about 1.6 mm. in outside diameter, and the largest eggs in the experiment, when corrected for shrinkage, measure $1.47+0.16=1.6$ mm. The egg diameters bounding each oocyte phase are only approximate, of course, but both dissected and sectioned ovaries were similarly fixed and cleared



TEXT-FIG. 4. Percentages of eggs measured for each experimental group of *Fundulus confluentus* found in oocyte phases II to V, respectively. For each group 100% represents the grand total of the largest 50 eggs per fish, *e. g.* 500 eggs for the December 15 control group of 10 fish.

so that their egg diameters could be compared. The small number of eggs approaching final maturity in the most mature fish of the experiment is consistent with the fact that we have been unable so far to strip and fecundate more than 25 eggs from a single female at one time.

Within each high-temperature group, a line of demarkation separates fish advanced in maturity from those retarded, with minimal overlap between the vanguard eggs of retarded fish, which are predominantly in phases II-III, and those of advanced fish, which are predominantly in phases IV-V. This effect is ascribable to high temperature, since no such separation occurs in the other groups, not even among the January 31 controls, although they exhibit the same range of oocyte phases. The two groups at high temperature are seen to differ from each other in amplitude and extent but not in character of response. The ratio of high-temperature fish with ovaries in stages III versus V is 13 : 2 in the long-day group and 5 : 9 in the short-day group (Table 3 and Text-fig. 3). The difference between these group responses (proportions) is significant. With Yate's correction, the value of Chi Square is such that $P<0.016$, and would doubtless be found somewhat less if computed by the laboriously exact method for 2×2 contingency tables. Table 3 shows in detail the amplitude of the response of each fish of these two groups, but the great advance in maturity of the short-day, high-temperature group over all others may be more clearly apprehended from Text-fig. 4.

In Text-fig. 4, the percentages of the largest 50 eggs per fish falling into each of the four oocyte phases (II-V) adds up to 100% for each experimental group. This permits group-to-group comparisons undistracted by the staggered responses of the individual fish and with a minimum of distortion from the moderate variation in numbers of fish per experimental group (*cf.* Table 2). It sums up the conformity of the two groups at low temperature, both showing no daylength influence, an accelerated transition from oocyte phase II to III-IV, and a lack of transition from phase IV to V. It registers the retardation at high temperature of the transition from oocyte phase II into III as well as the opposing accelerated progression toward definitive maturation in the larger eggs. The advance of the short-day, high-temperature group over all others is as unmistakable as it was unexpected. It clearly involves the concentration of vanguard eggs in oocyte phases IV and V, whereas in all other groups at the end of the experiment, including the final controls, the vanguard eggs are concentrated in oocyte phase III.

V. Discussion

Since the present experiment, with one partial exception (*vide infra* Hubbs & Strawn, 1957), is the only one of its kind conducted at so low a latitude, comparison of its results is virtually limited to those obtained with fishes from high latitudes. The high-latitude fishes studied spawn toward either end of that segment of the year occupied by the prolonged spawning season of low-latitude fishes, *viz.* one species in autumn, the rest in spring-summer. There is presumptive evidence that one spring

spawner, *Esox americanus vermiculatus,* bred again during an unseasonably warm autumn (Lagler & Hubbs, 1943), but fry from such adventitious spawning could not be expected to add effectively to the population. There is no reason, however, to minimize the biological importance of spawning by *F. confluentus* late in the season, for its eggs, even when stranded by receding waters toward the end of its spawning season, hatched when reflooded after having lain on sods out of water two to three months during an unusually cold winter (Harrington & Haeger, 1958; Harrington, 1959a). This projection of hatching far into the non-breeding season has been recorded only in other cyprinodont fishes in India, Africa and South America.

Baggerman (1957) found that four combinations of high or low temperature and long or short days induced in *Gasterosteus aculeatus* one pattern of positive and negative responses when applied to fish in an early phase of maturity and another pattern when applied to fish in a later phase of maturity. These phases are predicated on terminal behavioral criteria unaccompanied by gonad examination (*cf.* Harrington, 1959b), and have only general relevance here, but in so far as they furnish evidence of different responses by the reproductive mechanism to identical sets of extrinsic factors according to phase of maturity, they are of interest with respect to the different effects on earlier versus later oocyte phases found in the present study. Verhoeven & van Oordt (1955) discovered in *Rhodeus* a somewhat analogous difference in reaction to daylength and temperature according to phase of sexual maturity (cycle).

The inhibitory effect of high temperature on ovogenesis within earlier oocyte phases of *F. confluentus* finds a close parallel in the ovaries of *Apeltes* (Merriman & Schedl, 1941), *Phoxinus* (Bullough, 1939, graphs 10-11, not text; *cf.* Harrington, 1959b), and *Enneacanthus* (Harrington, 1956). In all experiments on other species, except *Notropis bifrenatus,* no attention was paid to the successive phases of ovogenesis, so that this reaction would have been overlooked. In the experiments on *N. bifrenatus,* however, all fish were kept at high temperature (Harrington, 1950, 1957). On short days, the eggs of *N. bifrenatus* increased in size up to a critical diameter, at which they stopped and beyond which they continued to final maturity outside the normal breeding season only with long days. Eggs reached the critical diameter sooner with long than with short days, and possibly at low temperature would have advanced even faster, but none began to exceed the critical diameter until mid-November. After this, long days at high temperature induced completion of the sex cycle (spawning) in six weeks, and when first imposed on other individuals January 1, they again induced spawning in six weeks. The effect of high temperature on the spawning of the low-latitude fish, *Etheostoma lepidum,* (Hubbs & Strawn, 1957), will be considered later in a more appropriate context.

The period before the end of which fishes respond neither to gonadotropin injection nor to environmental factors that otherwise induce spawning in the non-breeding season within a confined number of days has been called the refractory or postspawning period (*cf.* Atz, 1957; Harrington, 1959b). The refractory period seems to parallel that time interval during which the vanguard eggs are traversing some of the earlier oocyte phases. Present results, together with the ones cited above, suggest that in fishes this period can be shortened by subjecting them to that combination of external factors most favoring maturation through the early phases, after which the fish might be expected to respond at once to the different combination of external factors known to induce final maturation when imposed outside the natural spawning season. Both the critical egg diameter of *Notropis* and the disjunct distributions of the vanguard eggs of *Fundulus,* that were either retarded or accelerated in maturity by high temperature (Table 3 and Text-fig. 3), reflect a point below and above which there are different responses to a constantly maintained set of external conditions. This is intelligible, moreover, in the broader ecological context. It follows that both the term *refractory period* and any assertions that a given environmental variable is without influence on the sex cycle of a fish must be qualified as to the phases of maturation concerned.

If such a refractory period can be identified in *F. confluentus* at all, it can only be equated with the high-temperature retarded passage of oocytes from phase II (possibly also phase I) through III. Beyond phase III, the response to high temperature seems to change from negative to positive. Although eggs progressed to mid-phase IV at low temperature, at high temperature within the same time interval, those not kept from reaching phase IV continued through phase V toward final maturity (Tables 2-3 and Text-figs. 2-3). High temperature thus induced the later phases of maturity while retarding the earlier, and low temperature accelerated the earlier while arresting the later. There was no unmistakable influence by daylength at low temperature or on later phases of maturation at high temperature, but the retardation of

ovogenesis within the earlier oocyte phases by high temperature seems to have been strongly reinforced by long days (Table 3). It is worth mentioning here that in the European Pikeperch *(Lucioperca),* the transition from ovarian stage III to IV begins in October with the sudden fall in temperature (16.6° C. to 7.4° C.), lasts 1-1½ months, and is correlated with the resumption of rapid production and accumulation of gonadotropin (Trusov, 1947). The oocytes thereafter remain until January in the phase of primary accumulation of *fatty* yolk, which characterizes Trusov's ovarian stage IV-A, and was so named by him to distinguish it from the phase of primary accumulation of yolk as designated by other Russian authors, *i.e.* the phase of the formation of vacuoles or yolk vesicles (our phase III), which precedes it.

The refractory period of fishes is one of two alternate phases of an internal rhythm, even though its duration is conditioned by external factors. In *Notropis bifrenatus,* it begins long before temperature and daylength have declined to the levels at which they initiated breeding at the outset of the spawning season (Harrington, 1957). In *Gasterosteus aculeatus* under constant high temperature and long days there is an intrinsic 200-day reproductive rhythm, within which a reproductive period alternates with a non-reproductive (refractory) period (Baggerman, 1957). After the refractory period ends, breeding can be induced out of season by imposing a certain combination of external conditions (Harrington; Baggerman, *loc. cit.; et alii*), and the present results suggest that the refractory period can be shortened by imposing another different combination. Baggerman showed that in *Gasterosteus* there is no internal factor alone able to initiate a breeding period in the absence of an appropriate combination of external factors, but Bullough (1941) inferred an internal rhythm in *Phoxinus* capable of acting in the absence of the seasonal environmental conditions normally regulating the precise time of its action. When daylength was restricted starting early in the calendar year, *Phoxinus phoxinus,* which requires long days for maturation out of season, although retarded, still reached full breeding condition within its natural spawning season.

The over-all duration of spawning by *F. confluentus* populations at the same latitude is probably a matter of environmental regulation chiefly, although co-action of internal rhythm and external factors might be expected to delimit the spawning periods of individual fish in different ways. For example, the termination of the spawning period of a fish maturing early in the long spawning season of the species might be predominantly conditioned by its internal rhythm, as seems to be the case with *Notropis,* whereas that of a fish maturing late in the season could be the immediate result of the drop in temperature. The length of the over-all spawning season entails such a range in ages within the population that the internal rhythms of different fish would be out of phase much of the year unless synchronized by environmental factors within the spawning season, as well as by those of the non-breeding season, which appears to begin with the advent of subliminal low temperature.

It is inconceivable that the suppression of early phases of maturation by high temperature, alone or reinforced by long days, could be more than a retardation, for otherwise breeding would be prevented during most of the natural spawning season. The strong suppression of later phases of maturation by subliminal low temperature is understandable. A mere retardation of earlier phases of maturation by high temperature reinforced by long days, however, makes it possible to suppose that during the prolonged spawning season of *F. confluentus* the frequency or amplitude of oviposition, or both, as well as the numbers of spawners, may be greater toward the beginning and the end of the spawning season, when temperatures are lower and days are shorter. This combination of external factors has been shown to condition the spawning of salmonids in autumn (Hoover & Hubbard, 1937; *et alii*), but the influence of the same combination on the earliest spring spawners at high latitudes has not been studied experimentally. The above hypothesis could be tested statistically by sampling the gonosomatic indices of large numbers of female *F. confluentus* at intervals throughout the year. The problem is complicated, however, by (1) the probability that first spawners are recruited into the breeding population throughout the long spawning season, as they successively mature from the consequently staggered broods, some even displaced outside the spawning season through delayed hatching of stranded eggs, and by (2) the possibility that spawning may be partly tide-controlled (Harrington, 1959a), although tides are extremely variable in the Indian River region, where the present experimental material was obtained.

The field observations and experiments of Hubbs & Strawn (1957) on effects of daylength and temperature on the number of spawnings, number of eggs per spawning, and length of intervals between spawnings by the Greenthroat Darter, *Etheostoma lepidum,* provide the only other data cogent here concerning environmental influences on fish reproduction at low latitude.

Greenthroat Darters, living near springs at about 30° North Latitude where they were subject to an annual temperature range of 10° C. (14°-24° C.), when sampled throughout the year, were found in breeding condition on all dates of all but 1-2 months. Ripe fish were fewest in July, and young fish were lacking only in August. Downstream temperature extremes were greater (7°-35° C.), and where the annual range was 21.5° C., ripe fish were absent from May through October, presumably when temperatures exceeded 24° C. Experiments indicated an upper limit of egg production at 24°-27° C. These observations are suggestive with regard to the hypothesis that the reproduction activity of *F. confluentus* may slacken during the warmer, long-day, middle portion of its spawning season. Hubbs & Strawn found no evidence of a daylength influence on the amplitude or frequency of spawning. As they specify, however, the temperature was out of control and at or near the upper limit for egg production in their first series of experiments. Moreover, it is difficult to understand the rationale or to assess the results of subjecting the same fish to one daylength-temperature combination for 15 days and then to a different combination for 15 days. Since Greenthroat Darters spawn throughout all but the hottest, long-day months of the year, it might be supposed that the influence of daylength is at best subsidiary to that of temperature and possibly confined to reinforcing a retardation of ovogenesis by high-temperature extremes, as seems to be the case with *F. confluentus*. The demonstration of such a daylength effect may require an analysis of oocyte phases besides a stricter control of temperature, in view of the short annual range of temperatures to which these fish are habituated. Hubbs & Strawn made daily counts of eggs deposited on the sides of aquaria and on glass wool, but the count varied according to size of female, and egg eating was not controlled. Their second series of experiments was better controlled and of longer duration, and was evaluated in terms of variation in length of interspawning intervals, which these authors regarded as a more reliable criterion. Unfortunately there were few replications per daylength-temperature combination. Until these experiments are repeated with more refined techniques of control and evaluation, the results can be treated only as presumptive negative evidence of daylength influence on the spawning of Greenthroat Darters.

In *Fundulus confluentus,* the response of the gonosomatic index to maturation was mainly adterminal. It scarcely registered the advance of ovogenesis until the vanguard eggs were well into oocyte phase IV (*vide supra* and Text-fig. 3). It reflected maturation through the preceding oocyte phases only indirectly, as a time-measured end result. Earlier oocyte phases are important ecologically, however, and in different fishes are correlated with environmental factors in different ways. For instance, most high-latitude fishes winter over with ovaries in stage IV, but *Gasterosteus* does so with its ovaries in stage III (Kazanskii, 1951). Meien (1939) generalizes that at the end of the spawning season, the ovaries revert from stage VI to III in fishes with a long (interrupted) spawning season, but to stage II in fishes with a short (uninterrupted) one. This means that the post-spawning ovary of the former category is left with eggs in oocyte phases I-III, and that of the second with eggs in oocyte phases I-II only. Seasonal ovarian regression in the syrt', *Vimba vimba* L. var. *typicus,* for example, includes resorption of phase III oocytes (Sakun, 1957, figure 2), but in many other fishes, the eggs of this oocyte phase presumably are not resorbed at the end of the spawning season.

Although the above categories do not seem to accommodate *F. confluentus,* they attest to the ecological importance of earlier oocyte phases. In *F. confluentus,* ovarian maturation stage III is of peculiar interest because its vanguard eggs are in oocyte phase III, just before which the nucleoplasmic index begins to drop, within which the nucleus progressively diminishes in size and vitellogenesis begins, and at the end of which the nucleus stops shrinking and there is a change in the response of the oocytes to high temperature, *i.e.* they are no longer retarded in developing by sustained high temperature reinforced by long days but proceed rapidly to final maturity. The drop in the nucleoplasmic index and the reduction in nuclear diameter, which possibly involve emission into the cytoplasm of vitellogenic nuclear substances, leads inquiry from external environmental influences to levels of response among and within the cells of the target organ.

Summary and Conclusions

1. Specimens of Marsh Killifish, *Fundulus confluentus,* indigenous to 27° North Latitude, were subjected within the non-breeding season to four combinations of constant temperature (15 ± 1° C. or 30 ± 1° C.) and daylength (7 hours or 15 hours) for 45 days (December 15 to January 31).

2. The four experimental aquaria at the end

of the experiment contained 15, 17, 15 and 14 females, respectively, and 8-10 males each.

3. The effects of the variant treatments were measured in terms of the progress of the largest 50 eggs per fish through oocyte phases II to V. These four oocyte phases are defined by successive egg-diameter ranges. During phases II to IV, inclusive, the nuclear diameter was found to increase rapidly, gradually decrease, and then level off, respectively. As the eggs approach final maturity (phase V) the nucleus becomes obscured by yolk, and can no longer be measured in cleared eggs.

4. Oocyte phase II is the last phase of the primary (lesser) growth period, or the growth period 1 of authors. Oocyte phase III begins with the onset of vitellogenesis and is the first phase of the secondary (greater) growth period, or the growth period 2 of authors. It is the phase of primary accumulation of yolk, in the form of yolk vesicles (cytoplasmic vacuoles). Oocyte phase IV is the phase of accumulation of yolk in the form of lipid granules. Oocyte phase V comprises the stages of yolk consolidation and includes the definitive mature egg.

5. Each oocyte phase corresponds approximately with the phase of the most mature oocytes in ovaries belonging to the like-numbered ovarian maturation stage of Russian authors.

6. High temperature induced the later phases of maturation (oocyte phases IV through V), but retarded the earlier ones (II through III). Low temperature accelerated the earlier phases (II to mid-IV), but suppressed the later ones (mid-IV through V).

7. There was no incontrovertible evidence of daylength influence at low temperature or on later phases of maturation (oocyte phases IV through V) at high temperature, but the retardation of ovogenesis (oocyte phases II through III) by high temperature seemed to be strongly reinforced by long days.

8. The gonosomatic index scarcely reflected ovarian maturation until the largest 50 eggs were progressing from oocyte phase IV through V to final maturity.

9. The so-called refractory period, regarded as one of two alternate phases of an intrinsic reproductive rhythm in fishes, seems to parallel the passage of oocytes through the earlier oocyte phases, and nevertheless to be subject to external influence.

10. Because of the long breeding season (February into October) with its long-day, high-temperature middle portion, the retardation of early ovogenesis by high temperature reinforced by long days suggests that either or both the amplitude of oviposition and the number of spawners may be greater toward each end of the spawning period, when temperatures are lower and days are shorter. This hypothesis remains to be tested in the field.

11. The critical nature of oocyte phase III is indicated by the following: (a) the mean nuclear diameter shows consistently rapid increase prior to phase III, (b) the nucleoplasmic index drops abruptly just before phase III, (c) the mean nuclear diameter gradually decreases progressively throughout phase III, but levels off thereafter, (d) oocyte growth proceeds evenly and slowly up through phase III, accelerated by low temperature though unaffected by daylength at low temperature, but retarded by high temperature and still further retarded by long days in conjunction with high temperature, (e) maturation beyond phase III shows no evident responsiveness to daylength, advancing swiftly toward definitive maturity at high temperature, but not surpassing mid-phase IV at low temperature. These relationships acquire added significance from recent evidence favoring the hypothesis that in newt oocytes there is protein migration from cytoplasm to nucleus before vitellogenesis and in the opposite direction during vitellogenesis, and from the recent demonstration, in mouse cells, of nucleolar extrusion apparently related to the synthesis of material in the cytoplasm.

Acknowledgments

The author sincerely thanks Drs. James W. Atz, Charles M. Breder, Jr., Grace E. Pickford and Maurice W. Provost for their invaluable criticism and discussion of the manuscript and for many fruitful suggestions, and Dr. Lester R. Aronson for earlier helpful consultations. For resourceful execution of the illustrations and for faithful collaboration on the arduous measuremnts and computations, he is indebted, respectively, to Mr. Willem Janse and to Mr. Edward H. Everett, both on the staff of the Entomological Research Center, Florida State Board of Health.

Literature Cited

Atz, J. W.

1957. The relation of the pituitary to reproduction in fishes. Pp. 178-270, 372-482, 16 tables, in "The Physiology of the Pituitary Gland of Fishes," by G. E. Pickford & J. W. Atz. New York: New York Zoological Society. xxiii + 613 pp.

Baggerman, B.

1957. An experimental study of the timing of breeding and migration in the three-spined

stickleback (*Gasterosteus aculeatus* L.). Arch. néerl. Zool., 12: 105-317, 22 text-figs., 10 tables.

Bigelow, H. B., & W. C. Schroeder

1953. Fishes of the Gulf of Maine. U. S. Fish and Wildlife Service Fish. Bull., 53 (Fish. Bull. No. 74), viii + 577, 288 text-figs.

Bonhag, P. F.

1958. Ovarian structure and vitellogenesis in insects. Ann. Rev. Ent., 3: 137-160, 8 text-figs.

Brown, J. L.

1954. A review of the cyprinodont genus *Fundulus* of eastern United States. M. S. thesis, Cornell Univ. (Unpublished): 1-98, 25 figs., 33 tables.

1957. A key to the species and subspecies of the cyprinodont genus *Fundulus* in the United States and Canada east of the continental divide. J. Wash. Acad. Sci., 47 (3): 69-77.

Bullough, W. S.

1939. A study of the reproductive cycle of the minnow in relation to the environment. Proc. Zool. Soc. London, ser. A, 109 (1): 79-102, 7 tables, 17 graphs, 4 text-figs., 1 pl., 4 figs.

1941. The effect of the reduction of light in spring on the breeding season of the minnow (*Phoxinus laevis* Linn.). Proc. Zool. Soc. London, ser. A, 110 (3 and 4): 149-157, 1 graph, 2 text-figs., 1 pl., 3 figs. (1940).

Burger, J. W.

1939. Some experiments on the relation of the external environment to the spermatogenetic cycle of *Fundulus heteroclitus* (L.). Biol. Bull., 77 (1): 96-103, 2 pls.

1940. Some further experiments on the relation of the external environment to the spermatogenetic cycle of *Fundulus heteroclitus*. Bull. Mt. Desert Island Biol. Lab., 1940: 20-21.

Chopra, H. C.

1958. A morphological and histochemical study of the oocytes of the fish, *Ophiocephalus punctatus*, with particular reference to lipids. Quart. J. Micr. Sci., 99 (2): 149-158, 2 text-figs., appendix.

Clark, F. N.

1925. The life history of *Leuresthes tenuis*, an atherine fish with tide controlled spawning habits. Calif. Fish and Game Comm., Fish Bull., (10): 1-51, 6 pls., 17 figs., 6 graphs, 6 tables, 14 tables in appendix.

Detlaf, T. A., & A. S. Ginzburg

1954. "The Embryonic Development of Acipenserine Fishes (Sevriuga, Osetr and Beluga) in Connection with the Problems of Their Rearing." Inst. Morfol. Zhiv. A. N. Severtsov, Akad. Nauk. USSR, Moscow. 216 pp., 51 text-figs., 14 pls., 18 tables, 1 graph (folded). (In Russian).

Egami, N.

1956. Production of testis-ova in adult males of *Oryzias latipes*. VII. Seasonal changes of frequency of testis-ovum production. Jap. J. Zool., 12 (1): 71-79, 2 text-figs., 1 pl., 3 tables.

Ghosh, A., & A. B. Kar

1952. Seasonal changes in the gonads of the common Indian catfish, *Heteropneustes fossilis* (Bloch). Proc. Zool. Soc. Bengal, 5: 29-50, 3 text-figs., 1 pl., 8 figs.

Guthrie, M. J.

1928. Further observations on the ovarian eggs of *Fundulus*. Anat. Rec., 41 (1): 64-65 (Abstract).

1929. Cytoplasmic inclusions in the oocytes of vertebrates. Anat. Rec., 44 (3): 231-232 (Abstract).

Harrington, R. W., Jr.

1950. Preseasonal breeding by the Bridled Shiner, *Notropis bifrenatus*, induced under light-temperature control. Copeia, 1950 (4): 304-311, 1 text-fig.

1956. An experiment on the effects of contrasting daily photoperiods on gametogenesis and reproduction in the centrarchid fish, *Enneacanthus obesus* (Girard). J. Exp. Zool., 131 (2): 203-223, 1 text-fig., 1 pl., 8 figs., 4 tables.

1957. Sexual photoperiodicity of the cyprinid fish, *Notropis bifrenatus* (Cope), in relation to the phases of its annual reproductive cycle. J. Exp. Zool., 135 (3): 529-556, 4 text-figs., 1 pl., 3 figs., 1 table.

1959a. Delayed hatching in stranded eggs of Marsh Killifish, *Fundulus confluentus*. Ecology, 40 (3): 430-437, 4 text-figs.

1959b. Photoperiodism in fishes in relation to the annual sexual cycle. Pp. 641-667, 3 text-figs., in "Photoperiodism and Related Phenomena in Plants and Animals", edited by Robert B. Withrow. Washington: Amer. Assoc. Adv. Sci. Publ. No. 55. xvii + 903 pp.

Harrington, R. W., Jr., & J. S. Haeger

1958. Prolonged natural deferment of hatching in killifish. Science, 128 (3337): 1511.

Hoover, E. E., & H. E. Hubbard

1937. Modification of the sexual cycle in trout by control of light. Copeia, 1937 (4): 206-210, 2 figs., 1 table.

Hsu, T. C., & T. Y. Lou

1959. Nuclear extrusion in cells of Cloudman melanoma *in vitro*. Pp. 315-325, 12 text-figs., in "Pigment Cell Biology," edited by

Myron Gordon. New York: Academic Press. xiv + 647 pp.

Hubbs, C., & K. Strawn

1957. The effects of light and temperature on the fecundity of the greenthroat darter, *Etheostoma lepidum*. Ecology, 38 (4): 596-602, 2 text-figs., 2 tables.

Ivanov, M. F., & F. I. Dodzina

1957. "Histological analysis of the sex glands of Volga migrating herrings during the period of migrating and spawning." Pp. 155-180, 15 text-figs., in "Production of Fish Reserves in Connection with Hydroconstruction. Pt. 1." Uchenye Zapiski, Leningrad Gosud. Universitata, (228), Seria Biol. Nauk, 44. (In Russian).

Kazanskii, B. N.

1951. "Experimental analysis of the growth of the oocytes in fish." Dokl. Akad. Nauk, USSR, 80: 277-280, 2 text-figs., 2 tables. (In Russian).

Konopacka, B.

1935. Recherches histochimiques sur le développement des poissons. I. La vitellogénèse chez le Goujou (*Gobio fluviatilis*) et la carpe (*Cyprinus carpio*). Bull. Acad. polonaise Sci. et Let., ser. B, 2 (3): 163-182, 1 pl., 16 figs.

Kulaev, S. I.

1927. Beobachtungen über die Veränderungen der Hoden des Flussbarsches (*Perca fluviatilis* L.) im Laufe des Jahres. Zool. Zh., 7 (3): 50-53. (In Russian, with German summary).

1944. Structure and developmental cycle of testes in sexmature [*sic*] catfish (*Silurus glanis* L.). Zool. Zh., 23 (6): 330-341, 7 text-figs. (In Russian, with English summary).

Lagler, K. F., & C. Hubbs

1943. Fall spawning of the mud pickerel, *Esox vermiculatus* LeSueur. Copeia, 1943 (2): 131, 1 table.

Marshall, A. J., & B. Lofts

1956. The Leydig-cell homologue in certain teleost fishes. Nature (London), 177 (4511): 704-705, 3 text-figs.

Marza, V. D., E. V. Marza & M. J. Guthrie

1937. Histochemistry of the ovary of *Fundulus heteroclitus* with special reference to the differentiating oocytes. Biol. Bull., 73 (1): 67-92, 19 figs., 3 pls., 4 tables.

Mas, F.

1952. Contribution à l'histologie de l'oogénèse chez un téléostéen: *Perca fluviatilis* L. Bull. Soc. zool. France, 77 (5/6): 414-425, 3 text-figs.

Matthews, S. A.

1938. The seasonal cycle in the gonads of *Fundulus*. Biol. Bull., 75 (1): 66-74, 2 pls., 9 figs., 5 text-figs.

1939. The effect of light and temperature on the male sexual cycle in *Fundulus*. Biol. Bull., 77 (1): 92-95.

Meien, V. A.

1939. Le cycle annual des changements de l'ovaire des téléostéens. Izvest. Akad. Nauk, USSR (Moscow), Biol. ser.: 389-420, 18 text-figs. (In Russian, with French summary).

Merriman, D., & H. P. Schedl

1941. The effects of light and temperature on gametogenesis in the four-spined stickleback, *Apeltes quadracus* (Mitchill). J. Exp. Zool., 88 (3): 413-446, 5 text-figs., 2 pls., 8 figs., 1 table.

Miller, R. R.

1955. An annotated list of the American cyprinodontid fishes of the genus *Fundulus*, with the description of *Fundulus persimilis* from Yucatan. Occ. Pap. Mus. Zool., Univ. Mich., (568): 1-25, 1 pl., 2 figs., 4 tables.

Nath, V.

1957. Animal gametes. Part IV. Oogenesis. Res. Bull., Punjab Univ., India, 98: 145-200, 33 text-figs.

Nath, V., B. Singh & A. Bakr

1944. Fish oogenesis with particular reference to the so-called nucleolar extrusions. Proc. Nat. Inst. Sci. India, 10 (2): 247-253, 2 text-figs., 5 pls.

Pantelouris, E. M.

1958. Protein synthesis in newt oocytes. Exp. Cell Res., 14 (3): 584-595, 5 figs., 4 tables.

Sakun, O. F.

1957. "Analysis of the state of the sex glands of syrt' migrating through the Kegum fish pass." Pp. 181-191, 9 text-figs., in "Production of Fish Reserves in Connection with Hydroconstruction. Pt. 1." Uchenye Zapiski, Leningrad Gosud. Universitata, (228), Seria Biol. Nauk, 44. (In Russian).

Singh, B. N., & W. Boyle

1938. The vitellogenesis of *Gasterosteus aculeatus* (the stickleback) investigated by the ultra-centrifuge. Quart. J. Micr. Sci., 81 (1): n. s. 321: 83-106, 1 text-fig., 4 pls.

Strawn, K.

1958. Life history of the pigmy seahorse, *Hippocampus zosterae* Jordan and Gilbert, at Cedar Keys, Florida. Copeia, 1958 (1): 16-22, 4 text-figs., 3 tables.

SUBRAMANIAM, M. K., & R. G. AIYAR

1935. Oogenesis of *Acentrogobius neilli* (*Gobius neilli* Day), with special reference to the behaviour of the nucleoli. J. Roy. Micr. Soc., 55: 174-183, 2 pls., 16 figs.

TRUSOV, V. Z.

1947. "A histological analysis of the so-called stage IV of maturity of the ovaries of the sudak." Trud. Lab. Osnov Rybovodstva, 1: 155-167, 14 figs. (In Russian).

TURNER, C. L.

1919. The seasonal cycle in the spermary of the perch. J. Morph., 32 (3): 681-711, 44 figs.

VERHOEVEN, B., & G. J. VAN OORDT

1955. The influence of light and temperature on the sexual cycle of the bitterling, *Rhodeus amarus* (preliminary communication). Proc. Kon. Nedl. Akad. Wetsch., Amst., C, 58: 628-634, 2 text-figs., 1 table.

YAMAMOTO, K.

1955a. Studies on the formation of fish eggs. V. The chemical nature and the origin of the yolk vesicle in the oocytes of the herring, *Clupea pallasii*. Annot. Zool. Japon., 28 (3): 158-162, 2 pls., 21 figs.

1955b. Studies on the formation of fish eggs. VI. The chemical nature and origin of the yolk vesicle in the oocyte of the smelt, *Hypomesus japonicus*. Annot. Zool. Japon., 28 (4): 233-237, 2 pls., 21 figs.

1956a. Studies on the formation of fish eggs. I. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura*. J. Fac. Sci., Hokkaido Univ., ser. VI, Zool., 12 (3): 362-373, 2 text-figs., 3 pls., 26 figs.

1956b. Studies on the formation of fish eggs. II. Changes in the nucleus of the oocyte of *Liopsetta obscura*, with special reference to the activity of the nucleolus. J. Fac. Sci., Hokkaido Univ., ser. VI, Zool., 12 (3): 375-390, 4 text-figs., 3 pls., 21 figs.

1956c. Studies on the formation of fish eggs. III. Localization of polysaccharides in oocytes of *Liopsetta obscura*. J. Fac. Sci., Hokkaido Univ., ser. VI, Zool., 12 (3): 391-399, 2 pls., 17 figs.

1956d. Studies on the formation of fish eggs. IV. The chemical nature and the origin of the yolk vesicles in the oocyte of the flounder, *Liopsetta obscura*. Jap. J. Zool., 11 (5): 567-577, 3 pls., 25 figs.

1956e. Studies on the formation of fish eggs. VII. The fate of the yolk vesicle in the oocytes of the herring, *Clupea pallasii*, during vitellogenesis. Annot. Zool. Japon., 29 (2): 91-96, 1 pl., 9 figs., 1 table.

1956f. Studies on the formation of fish eggs. VIII. The fate of the yolk vesicle in the oocyte of the smelt, *Hypomesus japonicus*, during vitellogenesis. Embryologia, 3 (2): 131-138, 1 pl., 7 figs., 1 table.

1956g. Studies on the formation of fish eggs. IX. The fate of the yolk vesicle in the oocyte of the flounder, *Liopsetta obscura*, during vitellogenesis. Bull. Fac. Fish., Hokkaido Univ., 7 (3): 208-212, 1 pl., 9 figs.

1957. Studies on the formation of fish eggs. X. Demonstration of carbohydrates related to Da-Fano-positive bodies in the oocyte of the flounder, *Liopsetta obscura*. Annot. Zool. Japon., 30 (1): 33-36, 1 pl., 12 figs.

1958. Vitellogenesis in fish eggs. Symposia Cell. Chem., 8: 119-134, 2 pls., 17 figs. (In Japanese, with English abstract, pp. 131-132).

12

A Study of Lipids and Water of Liver and Muscle in *Fundulus heteroclitus* (Linnaeus) and *Stenotomus versicolor* (Mitchill)[1]

X. J. Musacchia

Department of Biology, St. Louis University

Introduction

The present study provides biochemical data for two common species from the temperate zone, the scup or porgy, *Stenotomus versicolor* (Mitchill), and the killifish or mummichog, *Fundulus heteroclitus* (Linnaeus). Although the role of tissue water and lipid composition has been considered (Gueylard, 1924, and Bloor, 1943), there does not appear to be any recent study of the subject in relation to fish tissues. The role of tissue lipids and their relationship to water content is appraised, and from a comparative viewpoint the data from a series of studies on arctic fishes have been used in discussion and evaluation in the present work.

Material and Methods

The facilities of the Marine Biological Laboratory at Woods Hole were used to obtain freshly-caught scup and killifish. Specimens were transferred to aquaria with fresh circulating sea water. Only active, vigorous specimens were selected for study. Each fish was spinalectomized at the base of the skull, and liver and muscle were excised in a matter of minutes. The pieces of tissue were removed from the same loci in every instance. Myotomic muscle of the scup was taken from a lateral area immediately posterior to the pectoral fin, and wedges of liver were taken from the same ventro-caudad marginal area each time. In the killifish, because of its small size, a slender strip of the left fillet was used for water analysis and a similar piece from the right fillet for fat extraction.

Tissue for water extraction was weighed on tarred pieces of aluminum foil and dried to constant weight. One hundred degrees centigrade for 22 hours, followed by two more weighings within six hours, was found to be sufficient to obtain a constant weight. From each scup, one to three pieces were weighed and dried. When more than one piece of tissue was taken from a given animal, the values were averaged. The dry weight method, because of its common usage, was selected in preference to others.

The methods used in the lipid analyses are identical with those cited in two earlier reports (Wilber & Musacchia, 1951, and Musacchia, Sullivan & Wilber, 1957).

Results

There is a notable similarity in the water content of the liver and the muscle from scup and killifish (Table 1). The water is in highest concentration in the muscle tissue of each species, and conversely the lipids are more concentrated in the liver. Although the cholesterol and total fatty acid concentration are highest in the liver of killifish, the ratio, cholesterol/total fatty acid, is almost identical for liver from both species: killifish, 0.080, and scup, 0.088. Table 1 shows that killifish liver has about twice as much total lipids or individual lipid components as scup liver. On the other hand, the phospholipid content of muscle from both species was found to be quite similar, in percent. of fresh tissue: killifish, 0.904, and scup, 0.951.

The summarized results (Table 1) suffice to illustrate in general differences in the two species, the killifish a euryhaline fish and scup a marine fish.

Discussion

Evaluation of the concentration of water in the tissues of fishes is essential to a better understanding of their biochemistry. Vinogradov (1953) devotes considerable attention to this

[1] This research was supported by a grant from the Permanent Science Fund of the American Academy of Arts and Sciences.

Table 1. Lipid Values for Liver and Muscle from Killifish and Scup
The values are percentages of fresh tissue; means and mean deviations are given.

Species	Cholesterol	Phospholipid	Total fatty acid	Total lipids	C/FA[1]	C/P[2]	H_2O
Stenotomus versicolor							
liver (18)[3]	0.386	3.345(19)	4.64	5.017(17)	0.088(17)	0.126(17)	73.372
	0.084	0.060	0.12	1.330	0.023	0.032	1.408
muscle (20)	0.091	0.951	2.03	2.121	0.051	0.096	79.299
	0.013	0.086	0.56	0.567	0.015	0.016	1.597
Fundulus heteroclitus							
liver (15)	0.799	6.972	10.14	10.909	0.080	0.107	71.458
	0.218	1.116	1.26	1.447	0.021	0.035	3.477
muscle (15)	0.133	0.904	1.60	1.732	0.085	0.170	78.998
	0.023	0.194	0.25	0.351	0.008	0.058	1.846

[1] C/FA = cholesterol/total fatty acid.
[2] C/P = cholesterol/phospholipid.
[3] Unless otherwise noted, the number of specimens analyzed is given in the first column.

subject. He lists values for the water content of muscle from 25 species of the Family Sparidae and, in general, the water content is about 75 percent. or less. The values range from 73.55 to 88.63 (in percent. of living matter) and, more specifically, for muscle of *Stenotomus argyrops,* 74.94 percent. and *Stenotomus chrysops* (= *S. versicolor*), 76.61 percent. In the present investigation, the mean value for water concentration in muscle of *Stenotomus versicolor* is 79.299 percent. This value is comparable with those of other members of the Family Sparidae; it is, however, slightly higher than the values reported by Vinogradov for the genus *Stenotomus.* Vinogradov expresses the view that in fish tissue the amount of water varies inversely with the amount of fat. The data obtained for scup and killifish tend to support this opinion. It is apparent (Table 1) that water is in greater concentration in muscle where total fatty acids are lower and, conversely, in liver total fatty acids are higher and water is in less concentration.

Some of the differences in the two species studied warrant discussion. For example, the liver tissue of killifish has almost double the cholesterol value of the liver of scup. The cholesterol content of muscle in killifish is in greater concentration in both absolute quantity and relationship to total fatty acid and phospholipid contents. According to some investigators, tissue cholesterol and tissue water are intimately associated. Mayer & Schaeffer (1914 a and b) reported a direct relationship between the ratio of cholesterol/total fatty acids and the water of imbibition. Cholesterol mobilization may be considered responsible for maintenance of normal water content of tissues in the euryhaline stickleback, *Gasterosteus pungitius,* (Gueylard, 1924). In his 1943 monograph, Bloor gives further discussion of the subject. It is evident from the data presented in Table 1 that there is a relative constancy in the water content of comparable tissues from scup and killifish, and at the same time there are gross differences in cholesterol content in the same tissues. These findings tend to weaken the hypothesis that the role of tissue cholesterol is to influence water concentration.

The relation of lipid phosphorus to cholesterol, *i.e.*, cholesterol/lipid phosphorus, has also been associated with the capacity of tissue to hold water (Mayer & Schaeffer, 1913, 1914 a and b). Again, from the evidence obtained, no correlation is apparent between the water content and the ratio, cholesterol/phospholipid. The concentration of phospholipid in muscle is similar in both species, but the ratio, cholesterol/phospholipid, is almost twice as great in *F. heteroclitus.* The gross hiatus in ratio values does not have a counterpart in the values for water content. With regard to the lipid ratios and the tissue water content values, these two species fail to provide evidence to support some of the contentions of Mayer & Schaeffer (*loc. cit.*).

Mayer & Schaeffer postulated that the *coefficient lipocytique,* cholesterol/total fatty acids, is constant in tissues from animals of diverse species. The values derived from the livers of scup and killifish, 0.088 (0.023) and 0.080 (0.021) respectively, are nearly the same, while those obtained for muscle are of the same order of magnitude, 0.051 (0.015) and 0.085 (0.008)

respectively. The data presented thus tend to confirm the reality of a lipocytic coefficient, and at least one other instance of supportive evidence can be found in earlier work where the muscle from *Boreogadus saida* (the arctic or polar tomcod) was reported as 0.088 ± 0.022 (Musacchia, Sullivan & Wilber, 1957).

Bloor (1943) shows that phospholipid values for muscle vary widely not only with the type of muscle but also with the animal. It is therefore of interest that the phospholipid values obtained for myotomic muscle of a variety of fishes possess some similarity: *Mallotus catervarius* 1.2, *Boreogadus saida* 1.7 (Musacchia, Sullivan & Wilber, 1957), *Myoxocephalus quadricornis* 1.85 (Musacchia & Clark, 1957), *Fundulus heteroclitus* 0.9, and *Stenotomus versicolor* 0.95 (all values expressed in percent. of fresh tissue). Some 30 years ago, muscle phospholipid values of the same order of magnitude were reported (in grams per hundred grams) for carp as 1.1 and dogfish as 1.1 (Javillier, *et al.*, 1928). These similarities are of value in further consideration of the role of phospholipid in muscle physiology.

Summary

1. Cholesterol, phospholipid, total fatty acid and water content of the liver and muscle of two common temperate zone fish, *Stenotomus versicolor* and *Fundulus heteroclitus*, have been determined, and the relationships of the various lipids to each other and to water content have been shown.

2. In both species, the muscle tissue has a higher water content than the liver, while the latter has the higher lipid concentration. An inverse relationship of tissue water and lipid concentrations is demonstrated.

3. Evidence is presented that tends to confirm the existence of a lipocytic coefficient, *i.e.*, that the cholesterol/total fatty acid ratio is constant in tissues from diverse species of fishes.

Literature Cited

Bloor, W. R.

1943. Biochemistry of the fatty acids and their compounds, the lipids. Reinhold, New York, 388 pp.

Gueylard, F.

1924. De l'adaptation au changements de salinité. Recherches biologiques et physicochemiques sur l'épinoche (*Gasterosteus leiurus* Cuv. et Val.) Vigot Frères, Ed. Paris.

Javillier, M. M., A. Crémieu & H. Hinglais

1928. Comparaison entre diverses espèces de vertébrés au point de vue des indices de phosphore nucléique et des bilans phosphorés de leurs organes. Bull. Soc. Chim. Biol., Vol. 10, pp. 327-337.

Mayer, A., & G. Schaeffer

1913. Recherches sur la teneur des tissues en lipoides. Existance possible d'une constance lipocytique. Jour. Physiol. et Pathol. Gen., Vol. 15, pp. 534-548.

1914a. Recherches sur les constantes cellulaires teneur des cellules en eau. I. Discussion théorique. L'eau, constante cellulaire. *Ibid.*, Vol. 16, pp. 1-16.

1914b. Recherches sur les constantes cellulaires teneur des cellules en eau. II. Rapport entre la teneur des cellules en lipoids et leur teneur en eau. *Ibid.* Vol. 16, pp. 23-38.

Musacchia, X. J., & M. Clark

1957. Effects of elevated temperatures on tissue chemistry of the arctic sculpin *Myoxocephalus quadricornis*. Physiol. Zool., Vol. 30, pp. 12-17.

Musacchia, X. J., Sullivan, B. J., & C. G. Wilber

1957. A comparison of liver and muscle lipids in arctic fishes. Copeia, Vol. 1957, pp. 10-12.

Vinogradov, A. P.

1953. The elementary chemical composition of marine organisms. Memoir. Sears Foundation for Marine Research. Yale University, New Haven, 647 pp.

Wilber, C. G., & X. J. Musacchia

1951. A survey of lipids in arctic animals. A. F. Technical Report. U.S.A.F. Wright Air Development Center, Wright-Patterson Air Force Base, Dayton, Ohio. No. 6463, pp. 20.

13

Ectyonin, an Antimicrobial Agent from the Sponge, *Microciona prolifera* Verrill

Ross F. Nigrelli, Sophie Jakowska & Idelisa Calventi

Department of Marine Biochemistry and Ecology, New York Aquarium, New York Zoological Society

(Plate I)

Introduction

It is generally recognized that external metabolites may play a regulatory role in aquatic ecology by either inhibiting or promoting growth (Nigrelli, 1958). Previous studies in the laboratory of Marine Biochemistry and Ecology have indicated that a number of marine invertebrates produce such substances. Holothurin, a toxic steroid saponin isolated by Nigrelli (1952) from the Bahamian sea cucumber, *Actinopyga agassizi* Selenka, is an example of such biologically-active material.

In work (unpublished) done in 1954 and 1955, the senior author found that simple water extracts of several species of Atlantic sponges were capable of inhibiting growth of certain marine Gram-negative bacteria and *Micrococcus aureus*. The degree of inhibition varied with the source of the active principle and other uncontrolled factors. The present report deals with a broad spectrum antimicrobial agent extracted from the red-beard sponge, *Microciona prolifera*, commonly found along the Atlantic coast of North America. Ectyonin, the name proposed for this substance, is derived from the name of the sub-family Ectyoninae.

Materials and Methods

Preparation of the Sponge.—The sponges were collected from July through November. Colonies, ranging from one to three feet in width and up to one foot in height (Fig. 1)[1], were quickly washed with sea water to remove superficial impurities; the inhabiting fauna (commensals and epibionts) was manually removed. The latter consisted of worms (*Clymenella, Lepidonotus, Spirorbis*), crustaceans (*Carcinides maenas, Panopeus, Balanus* and isopods), clumps of mussels (*Modiolus*), sea anemones (*Sagartia*), some bryozoans and gobioid fish. Some of the cleaned sponge was stored in a freezer wrapped in aluminum foil.

Extractions were made of fragments and homogenates of fresh and frozen sponges, and of oven-dried (over-night at 60-80° C.) material that was fragmented or pulverized. Sponges kept in running sea water for a few weeks were also extracted; the color of this material changed from bright brick red to purplish black, possibly indicating some decomposition.

Preparation and Purification of Extracts.—The following procedures were found most satisfactory under our laboratory conditions. Dried sponges were cut into small pieces or pulverized by mortar and pestle or Waring blendor. Extraction was made with ethyl ether for 24 hours at 7° C. The orange-colored extract was decanted and decolorized with Norit-A for approximately one hour at room temperature. The total solids were determined before and after decolorization, and the material was then concentrated by evaporation. Extracts concentrated without decolorizing were thick and viscid; such materials were difficult to decolorize.

Homogenates of fresh sponges were made with hot and cold distilled water, and fractionated by centrifugation at 10,000 to 15,000 rpm, or extracted with 95% ethanol, acetone, chloroform, benzene, petroleum ether and ethyl ether, and then centrifuged. Intractable emulsions were obtained in some cases. The water in ethyl ether extracts of living cell suspensions and fragmented and homogenized fresh sponges was

[1]These are considerably larger than those reported by Hartman, 1958.

removed with sodium sulfate. Large quantities of dried sponge were also extracted in Soxhlet with ethanol, chloroform and acetone, evaporated and re-extracted with a small volume of ethyl ether. Purification included removal of pigments with Norit-A, filtration and hydrolysis with cold N/100 NaOH.

The components of the ethyl extracts were chromatographed on paper with ethyl ether and petroleum ether used as ascending phases. Petroleum ether and acetone fractions of purified ether extracts were also collected from an alumina adsorption column (80-200 MM). Concentrated decolorized ether extracts of known antimicrobial activity were used to obtain crystals at room temperature and at 0° C.

Microbiological Testing Methods. — Filter paper discs 13 mm. in diameter were repeatedly impregnated with solutions to be tested, or smeared with semi-solid fractions, and then dried at room temperature. Control discs were impregnated with the solvents. The test organisms were *Micrococcus aureus, Escherichia coli, Pseudomonas pyocyanea, Klebsiella pneumoniae, Mycobacterium* from Cobra, *Mycobacterium* 607 (Bovine) and *Candida albicans.* Plates were poured with 1 ml. of a 24-hour culture in 20 ml. nutrient agar; discs were placed on the solidified medium and the plates incubated at 37° C. for 24-48 hours, and longer for *Mycobacterium* and *Candida.* Inhibition zones were recorded in mm., measuring from the edge of the discs. The chromatographic paper strip containing the components of the ethyl ether extract were also tested in this manner, as were discs impregnated with sesame oil suspensions of evaporated, decolorized, ethyl ether extracts. The latter material was also used for the *in vivo* tests.

In Vivo Tests for Toxicity and Antimicrobial Action.—The tests were limited to a few animals in view of the difficulties encountered in preparing sufficient amounts of ethyl ether extracts under our laboratory conditions. A suspension of the active material was made by evaporating 20 ml. of decolorized ether extract on 0.5 ml. of sesame oil. Paper discs dipped in this suspension were found to produce a 7 mm. zone of inhibition against *Pseudomonas pyocyanea.* Adult killifish, *Fundulus heteroclitus,* two inches long, were injected intraperitoneally with 0.02 ml. of the suspension; four fish were simultaneously injected with 0.02 ml. of a 24-hour-old broth culture of *Pseudomonas* and two fish were given the bacterial suspension only. Two hybrid AB mice were injected subdermally in the axillary region with 0.2 ml. of the suspension.

Observations

In vitro tests for antimicrobial activity were made of all extracts and their fractions. The active substance appears to be only slightly soluble in water, chloroform and acetone. It is best obtained with ethyl ether from living cell suspensions, fragments of fresh or frozen sponge, or from evaporated Soxhlet extracts of dried material. It has been found necessary to decolorize the extracts prior to reducing them in volume, even though the original pigment-containing ethyl ether extracts produced striking inhibition zones in all organisms tested (Fig. 2A). Discs impregnated with large amounts of the thick pigmented extracts failed to produce inhibition in proportion to the theoretically determined amounts of the inhibiting agent (Fig. 2D).

No quantitative data were obtained in the early phase of this work. The information in Table 1 was derived from analysis of sponges collected on specific dates. In each case extracts were made of oven-dried fresh and frozen sponges. Comparable weights of dry sponge were used; the original wet weight was approximately 180 g. Usually the dry weight represents ⅕th-⅙th of the wet weight of the sponge.

Table 1.

	Method of Storage			
	Frozen		Dry	
Date of collection	9/15/58	11/8/58	9/15/58	11/8/58
Dry weight (g.)	31.0	33.0	31.0	33.0
Total ether-extracted solids (mg.)	138.5	107.5	737.0	616.0
Total solids after decolorizing (mg.)	11.85	7.05	77.0	32.0

The *in vitro* activity was proportionate to the total solids per ml. of decolorized ethyl ether extract of sponge of the same collection (Table 2). Materials washed or handled too long before freezing and drying yielded extracts of low antimicrobial activity but the solids per ml. were higher. Although non-decolorized, water-free ethyl ether extracts of fresh sponge collected in July (7/21/58) produced zones of inhibition up to 27 mm., these results could not be repeated with sponge collected in the autumn. Decrease in the relative amounts of the antimicrobial substance was also obtained when the

sponge was allowed to start decomposing in running sea-water; extracts of healthy and such partly decomposed sponge produced inhibition zones of 10-14 mm. and 1.5-3 mm., respectively.

When the amount of ether-extractable decolorized solids present on a disc was estimated on the basis of total solids per ml. of extract, the results are shown in Fig. 3 and were as follows:

Table 2.

Date of Collection	Estimated Decolorized Solids/disc	Av. Inhibition
9/15/58	7.9 mg.	5.0 mm.
	3.9	2.0
11/8/58	4.7	4.5
	2.35	2.0

As shown in Table 1, sponges collected in September produce relatively more ether extractable solids than sponges obtained in November, although there seems to be considerable difference in the total solids obtained from frozen and dried material. This may be due to slight difference in the extraction method. From these and other data it appears that the solids in decolorized ether extracts represent from 0.04 to 0.10% of the dry weight of the sponge. Materials removable by cold NaOH treatment represents slightly over 90% of the solids in the decolorized ethyl ether extracts.

The decolorized ether extracts that showed antimicrobial activity produced a mixture of crystals when evaporated at room temperature; at 0° C. white crystals were formed on the strip of filter paper immersed in the container. No antimicrobial activity was demonstrated, but this may be due to the small amount of crystals collected in this manner.

Decolorized extracts treated with NaOH, or fractions collected on an alumina column, showed no antimicrobial activity. The extract distributed along the chromatographic paper strip produced a characteristic pattern of inhibition when applied to a seeded plate. The greatest zone of inhibition occurred on either side of the middle part of the strip and it tapered towards the starting and terminal points. The suspension of ethyl ether extract in sesame oil placed on the disc produced a 7 mm. zone of inhibition.

With the exception of one death due to injury, all fish injected with both bacteria and sesame oil suspension of the active extract survived. Fish injected with bacteria alone died in a relatively short time. One mouse developed a nodule at the site of injection after several weeks but otherwise showed no ill effects from the intradermal injection of the sesame oil suspension of the sponge extract. There was some indication that the fish infected with *Pseudomonas* may have been protected by Ectyonin. Further tests, however, are needed to evaluate the *in vivo* effects.

Discussion

Microciona prolifera is relatively abundant from early June through November along the coast of the western North Atlantic. Although no attempts were made during the early phase of this work to quantitate the active principle in this sponge, the data obtained from materials collected in September and November indicate that the yield decreases in the colder months. To what extent this may be related to the natural metabolic cycle of the sponge or to environmental conditions has not been determined.

The solids removable by cold NaOH treatment, and presumably representing over 90% of the total ethyl ether extractable solids after decolorizing, showed no antimicrobial properties in the *in vivo* tests. This may be due either to the extremely low yields after this procedure or to the possibility that the antimicrobial property is associated with the lipoid fraction. It should be mentioned here that *Microciona* contains a variety of sterols (Bergmann *et al.*, 1945).

The active principle in *Microciona prolifera* is remarkable for its activity against a variety of microorganisms, including Gram-positive, Gram-negative, and acid-fast forms, as well as *Candida albicans*. Of special interest is its activity against *Pseudomonas pyocyanea*. Under laboratory conditions, water extracts of *Microciona* were not significantly antimicrobial but it is suggested that under natural conditions Ectyonin may play some role in the biochemical ecology of the sponge.

Summary

Ectyonin, a fraction of the ethyl ether extracts of the red-beard sponge, *Microciona prolifera*, showed antimicrobial properties when tested *in vitro* on Gram-positive, Gram-negative, acid-fast bacteria, and *Candida albicans*. Preliminary tests indicate that it is not toxic to fish and mice.

References

Bergmann, Werner, Harold F. Schedl & Eva M. Low

1945. Contributions to the study of marine products. XVIII. Microcionasterol and other sterols of sponges. J. Org. Chem., 10 (6): 580-586.

HARTMAN, WILLARD D.

1958. Natural History of the Marine Sponges of Southern New England. Peabody Museum of Natural History, Yale Univ. Bull. 12, i-vi, 155 pp., 12 pls.

NIGRELLI, ROSS F.

1952. The effects of Holothurin on fish, and mice with Sarcoma 180. Zoologica, 37: 89-90.

1958. Dutchman's Baccy Juice or growth-promoting and growth-inhibiting substances of marine origin. Trans. N. Y. Acad. Sci., Ser. II, 20 (3): 248-262.

EXPLANATION OF THE PLATE

PLATE I

FIG. 1. Collection of large colonies of *Microciona prolifera* from the Long Island coast in late summer.

FIG. 2. **A.** Zone of inhibition of *Pseudomonas pyocyanea* produced by crude ethyl ether extract of unknown concentration from sponge collected in mid-summer. **D.** Disc impregnated with thick pigmented extract.

FIG. 3. Zones of inhibition produced by known amounts of solids in decolorized ethyl ether extracts prepared from dried sponge. Material collected in September.

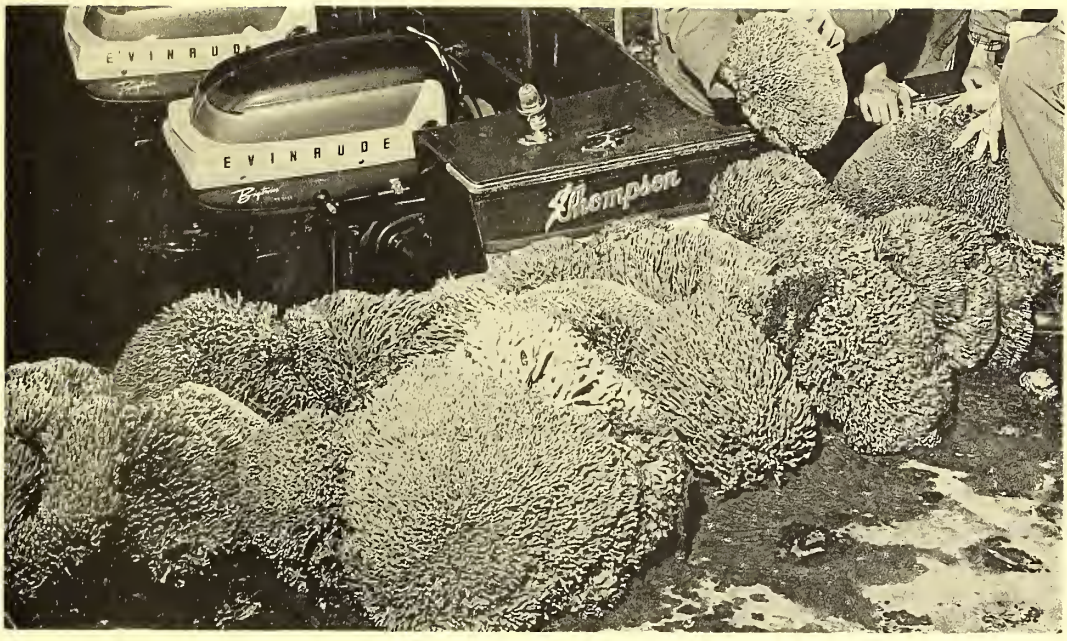


FIG. 1

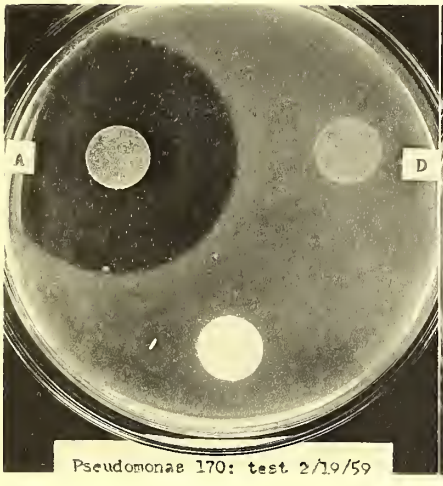


FIG. 2

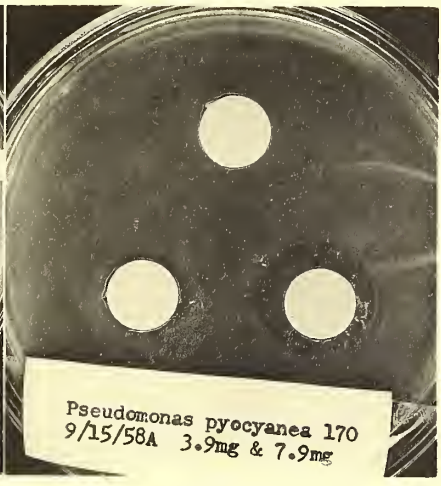


FIG. 3

ECTYONIN, AN ANTIMICROBIAL AGENT FROM THE SPONGE,
MICROCIONA PROLIFERA VERRILL

INDEX

Names in **bold face** indicate new genera, species or subsjecies; numbers in **bold face** indicate illustrations; numbers in parentheses are the series numbers of papers containing the plates listed immediately following.

NEW YORK ZOOLOGICAL SOCIETY

GENERAL OFFICE
30 East Fortieth Street, New York 16, N. Y.

PUBLICATION OFFICE
The Zoological Park, New York 60, N. Y.

OFFICERS

PRESIDENT	VICE-PRESIDENT	SECRETARY	TREASURER
Fairfield Osborn	Laurance S. Rockefeller	George Wall Merck	David H. McAlpin

SCIENTIFIC STAFF:

John Tee-Van ..... *General Director*
Christopher W. Coates. . *Director, Aquarium*

ZOOLOGICAL PARK

Joseph A. Davis, Jr.. . *Assistant Curator, Mammals*
Grace Davall..... *Assistant Curator, Mammals and Birds*
William G. Conway. . *Curator, Birds*
Herndon G. Dowling. *Associate Curator, Reptiles*
Charles P. Gandal .. *Veterinarian*
Lee S. Crandall..... *General Curator Emeritus*
William Beebe..... *Honorary Curator, Birds*

AQUARIUM

James W. Atz..... *Associate Curator*
Carleton Ray..... *Assistant to the Director*
Ross F. Nigrelli..... *Pathologist & Chairman of Department of Marine Biochemistry & Ecology*
Sophie Jakowska..... *Research Associate in Experimental Biology*
C. M. Breder, Jr..... *Research Associate in Ichthyology*
Harry A. Charipper... *Research Associate in Histology*
Homer W. Smith..... *Research Associate in Physiology*

GENERAL

William Bridges .. *Editor & Curator, Publications*
Sam Dunton ..... *Photographer*
Henry M. Lester. . *Photographic Consultant*

DEPARTMENT OF TROPICAL RESEARCH

William Beebe..... *Director Emeritus*
Jocelyn Crane..... *Assistant Director*
David W. Snow ..... *Resident Naturalist*
Henry Fleming ..... *Entomologist*
John Tee-Van ..... *Associate*
William K. Gregory.... *Associate*

AFFILIATES

L. Floyd Clarke ..... *Director, Jackson Hole Biological Research Station*

SCIENTIFIC ADVISORY COUNCIL

A. Raymond Dochez
Alfred E. Emerson
W. A. Hagan
Caryl P. Haskins
John S. Nicholas

EDITORIAL COMMITTEE

Fairfield Osborn, *Chairman*
James W. Atz
William Beebe
William Bridges
Christopher W. Coates
William G. Conway
Lee S. Crandall
John Tee-Van